Drinking-Water Nitrate and Incidence of Congenital Anomalies in the Annapolis Valley, Nova Scotia

by

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TABLE OF CONTENTS

Page

List of Tables viii

List of Figures x

List of Maps xi

Abstract xii

List of Abbreviations and Symbols Used xiii

Acknowledgements xiv

Chapter 1: Introduction 1

1.1 Background and Relevance 1

1.2 Literature Review 2

1.2.1 Congenital Anomalies in Nova Scotia 2

1.2.2 Etiology of Congenital Anomalies 2

1.2.3 Nitrate and Nitrite: Sources and Prevalence in the Environment 3

1.2.4 Spatial Variability of Groundwater Nitrate 5

1.2.5 Temporal Variability of Groundwater Nitrate 6

1.2.6 Nitrates and Human Health 8

1.2.7 Nitrates and Congenital Anomalies 8

1.2.8 Limitations in Existing Research on Nitrates and Congenital Anomalies 11

1.2.9 Exposure Assessment in Research on Drinking-Water and Pregnancy Outcomes 15

1.2.10 Exposure Assessment Using GIS 17

Chapter 2: Hypothesis 20

Chapter 3: Methodology 21
3.1 Study Design 21
3.1.1 Inclusion Criteria 22
3.1.2 Exclusion Criteria 22
3.2 Study Area 23
3.3 Study Population 25
3.4 Health Data Sources 26
3.5 Water Quality and Nitrate Data Sources 27
3.6 Data Analysis 28
3.6.1 Variables Included in the Study 28
3.6.2 Data Analysis of Health Variables 29
3.6.3 Descriptive Statistics for Water Quality and Nitrate Data 29
3.6.4 Exposure Assessment 30
3.6.5 Interpolating Nitrate Concentrations in Rural Areas 31
3.6.6 Delineating Municipal Water Distribution Systems 33
3.6.7 Combining Rural and Municipal Nitrate Concentration Maps 33
3.6.8 Linking Addresses to Water Quality Information 34
3.7 Statistical Modelling 35
3.8 Data Access and Ethical Review Process 36
Chapter 4: Results 37
4.1 Participant Selection 37
4.2 Results of Water Quality Data and Nitrate Data Analysis 38
4.2.1 Descriptive Statistics for Municipal and Rural Data 38

4.2.2 Linear Mixed Effects Models of Nitrate Concentrations From Municipal Water Supplies 39

4.2.3 Linear Mixed Effects Models of Nitrate Concentrations from Rural Wells 40

4.2.4 Exposure Assessment 40

4.3 Descriptive Statistics for Health Data 42

4.3.1 Frequencies of Congenital Anomaly Diagnoses 42

4.3.2 Distributions and Univariate Associations for All Variables Included in the Study 42

4.3.3 Distribution of Covariates Among Cases Stratified by Maternal Age at Conception 44

4.4 Statistical Modelling 44

4.4.1 Models Including All Study Participants 44

4.4.2 Subset Models for Cases and Controls Using Groundwater 46

4.4.3 Subset Models for Cases and Controls Using Municipal Water or Rural Wells 46

4.4.4 Subset Models For Cases and Controls Conceived Before or After the Fortification of Grain with Folic Acid in 1998 47

Chapter 5: Discussion 48

5.1 Major Findings 48

5.1.1 Water Quality and Nitrate Data Analysis 48

5.1.2 Exposure Assessment 49

5.1.3 Descriptive Statistics for Health Data 50

5.1.4 Statistical Modelling 52

5.1.5 Statistical Modelling of Demographic Characteristics 53

5.1.6 Statistical Modelling of Maternal Risk Factors 55

5.1.7 Statistical Modelling of Water Quality Variables and Nitrate 59

5.2 Study Strengths 61

5.3 Study Limitations 63

5.4 Policy Implications 68

5.5 Future Research 70

Chapter 6: Conclusion 73

References 75

Appendix 1: List of Major Anomalies by Body System as Defined by the Reproductive Care Program of Nova Scotia 106

LIST OF TABLES

Page

Table 1. Descriptive statistics of nitrate measurements from municipal water supplies in the Annapolis Valley study area and Halifax Regional Municipality from 1996 to 2003 (all concentrations represent nitrate-nitrogen in mg/L). 86

Table 2. Prediction errors from various interpolation models of rural well nitrate concentrations (n=1113) from July 1999 to February 2000 in the Annapolis Valley and the associated model options. 87

Table 3. Frequency of diagnoses of major congenital anomalies in the Annapolis Valley, categorized by body system, over the entire study period (1987-2006), during the period prior to the fortification of food in Canada with folate (1987-1997), and for the period after folate fortification (1998-2006). 88

Table 4. Distribution of variables between cases of congenital anomalies and controls from the Annapolis Valley, as well as univariate associations between incidence of congenital anomalies and all variables for cases and controls (1987-2006). 89

Table 5. Distribution of variables between cases of congenital anomalies from the Annapolis Valley and controls from the Halifax Regional Municipality, as well as univariate associations between incidence of congenital anomalies and all variables for cases and controls (1988-2006). 90

Table 6. Distribution of maternal demographic characteristics, risk factors, water quality variables and nitrate by maternal age group among cases of congenital anomalies (n=606) in Annapolis Valley (1987-2006). 91

Table 7. Multivariable associations between incidence of congenital anomalies and all variables for cases and controls from the Annapolis Valley (1987-2006). 92

Table 8. Multivariable associations between incidence of congenital anomalies and all variables (except folic acid supplementation and pre-pregnancy weight) for cases and controls from the Annapolis Valley (1987-2006). 93

Table 9. Multivariable associations between incidence of congenital anomalies and all variables (except water quality and nitrate) for cases from the Annapolis Valley and controls from the Halifax Regional Municipality (1987-2006). 94

Table 10. Multivariable associations between incidence of congenital anomalies and all variables (except water quality, nitrate, folic acid supplementation and pre-pregnancy weight) for cases from the Annapolis Valley and controls from the Halifax Regional Municipality (1987-2006). 95

Table 11. Multivariable associations between incidence of congenital anomalies 96
and all variables (except folic acid supplementation and pre-pregnancy weight),
for all cases and controls from the Annapolis Valley, and for only cases and
controls from the Annapolis Valley that have groundwater sources of drinking-water
(1987-2006).

Table 12. Multivariable associations between incidence of congenital anomalies 97
anomalies and all variables (except folic acid supplementation and pre-pregnancy
weight), for all cases and controls from the Annapolis Valley, only cases and
controls from the Annapolis Valley that live in municipal areas, and only cases
and controls from the Annapolis Valley that live in rural areas.

Table 13. Multivariable associations between incidence of congenital anomalies 98
and all variables (except folic acid supplementation and pre-pregnancy weight), for
cases and controls from the Annapolis Valley conceived prior to folate fortification
(1987-1997), and for cases and controls from the Annapolis Valley conceived after
folate fortification (1998-2006).

LIST OF FIGURES

Page

Figure 1. A histogram displaying nitrate concentrations (as nitrate-nitrogen) from all samples taken from rural wells in the Annapolis Valley study area from July 1999 to February 2000 (n=1113). 99

LIST OF MAPS

Page

Map 1. The boundaries of the water distribution systems of municipalities with public water supplies within the Annapolis Valley study area. 100

Map 2. The locations of 140 private wells in the Annapolis Valley study area between July 1999 and February 2000. 101

Map 3. Groundwater nitrate concentration estimates in the Annapolis Valley study area derived from an ordinary kriging interpolation of the nitrate concentrations measured from 140 private wells in the study area. 102

Map 4. Groundwater nitrate concentration estimates in the Annapolis Valley study area derived from an ordinary kriging interpolation of the nitrate concentrations measured from a randomly selected 90% of the 140 private wells in the study area. 103

Map 5. Drinking-water nitrate concentration estimates in the Annapolis Valley study area derived in rural areas from an ordinary kriging interpolation of the groundwater nitrate concentrations and derived in municipal areas from the median drinking-water nitrate concentrations of public water supplies. 104

Map 6. The direction of groundwater flow in the Annapolis Valley study area and the shape of neighbourhood divided into quadrants from which neighbours were selected for the ordinary kriging interpolation. 105

ABSTRACT

This study sought to determine if there is an association between drinking-water nitrate exposure and incidence of congenital anomalies in a study area within the Annapolis Valley, Nova Scotia. Cases and controls were selected for the period 1988-2006 from the Nova Scotia Atlee Perinatal Database and the Fetal Anomaly Database, province-wide enhanced perinatal databases. In rural areas, ordinary kriging was used to interpolate groundwater nitrate concentrations from 140 private wells. Nitrate concentrations from public water supplies were used to estimate exposure in municipal areas. After controlling for demographic traits and other known risk factors for congenital anomalies available in the databases, a non-significant positive association between the incidence of congenital anomalies and exposure to drinking-water nitrate > 1 mg/L was observed (OR = 1.65, 95% CI 0.83-3.27 for 1-5.56 mg/L; OR= 1.66, 95% CI 0.81-3.42 for > 5.56 mg/L). After stratifying the data according to conception before or after the folic acid fortification of food in Canada and the inception of a province-wide standardized address system, which increased the proportion of fetuses diagnosed with congenital anomalies eligible for inclusion in the study, there was a significant positive association between congenital anomalies and drinking-water nitrate > 1 mg/L (OR=2.44, 95% CI 1.05-5.66 for 1-5.56 mg/L) for the period 1998-2006. The results of this study indicate that incidence of congenital anomalies may be increased upon exposure to drinking-water nitrate, even at concentrations below the Canadian Maximum Allowable Concentration (10mg/L). Prospective research should be undertaken to further explore these associations.

LIST OF ABBREVIATIONS AND SYMBOLS USED

NTD	Neural tube defects
GIS	Geographic Information Systems
MAC	Maximum Allowable Concentration
NSAPD	Nova Scotia Atlee Perinatal Database
FADB	Fetal Anomaly Database
NO_3	Nitrate
NO_2	Nitrite
NH_4	Ammonium
PEI	Prince Edward Island
CNS	Central Nervous System
MIZ	Metropolitan Influence Zone
CMA	Census Metropolitan Area
HRM	Halifax Regional Municipality
RCP	Reproductive Care Program of Nova Scotia
IDW	Inverse Distance Weighting
ADI	Acceptable Daily Intake

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CHAPTER 1: INTRODUCTION

1.1 Background and Relevance

Within Nova Scotia, incidences of neural tube defects (NTD), congenital heart defects and Down's syndrome are among the highest in Canada (1). Though many congenital anomalies are not severe, and some can be corrected, individuals with congenital anomalies, and their families, may experience substantial emotional and economic burdens (1). The etiologies of many congenital anomalies remain unknown and our current lack of understanding of the causes of congenital anomalies and their widespread health impacts warrants further investigation into risk factors, environmental causes and means of prevention (1).

The teratogenic potential of many chemical compounds has prompted concerns that environmental exposures may contribute to the development of those congenital anomalies with unknown etiologies (2). Several ecological and case-control studies have shown a positive association between drinking-water nitrate levels and incidence of congenital anomalies (3-7).

This research project aimed to overcome some of the limitations faced by previous studies and meet some of the recommendations given in recent literature. These recommendations included: studying users of private wells, and using Geographic Information Systems (GIS) to improve nitrate exposure estimates and examining how nitrate exposure relates to other risk factors for congenital anomalies (8,9).

This project focused on the agricultural region of the Annapolis Valley, Nova Scotia. Previous water-quality surveys have determined that nearly 20% of wells sampled in a study area within the Annapolis Valley have nitrate-nitrogen levels above the Maximum Allowable Concentration (MAC) of 10 mg/L (10,11), suggesting substantial prenatal exposure to nitrates in the area.

1.2 Literature Review

1.2.1 Congenital Anomalies in Nova Scotia

Between 2% and 3% of Canadian children are born with a congenital anomaly (1). Any abnormality in body structure, function or metabolism present at birth is considered a congenital anomaly (12). In 1995, 1.9 per 10,000 Canadian live births resulted in death due to a congenital anomaly (1). Case fatality rates for certain anomalies, such as trisomies 13 and 18, as well as anencephaly, can reach 100% by a child's first birthday (1).

1.2.2 Etiology of Congenital Anomalies

Much of the etiology of congenital anomalies remains unknown; approximately 40-60% of congenital anomalies have unexplained causes. About 15-20% of congenital anomalies have well-defined genetic causes and approximately 20-25% have multifactorial causes whereby genetic make-up, environmental exposures and interactions between genes and the environment each contribute to interference with normal embryonic development (1). It has been suggested that teratogens in the environment independently cause 8-12% of congenital anomalies (1). Some drugs, notably thalidomide, certain anti-depressives and

folic acid antagonists, as well as alcohol, nutrient deficiencies and components of drinking-water, namely trihalomethanes and nitrates, have been linked with congenital anomalies (1,3,5,7).

1.2.3 Nitrate and Nitrite: Sources and Prevalence in the Environment

Nitrogen is required for the function of living organisms, though most of Earth's nitrogen is bound in the form of N_2, which is biologically unavailable (13). The primary forms of biologically available nitrogen are nitrate (NO_3), nitrite (NO_2) and ammonium (NH_4). All three are created through the processes of nitrogen fixation by microbial action in leguminous plants and bacterial nitrification (14).

Anthropogenic sources of fixed nitrogen constitute an excess of 50-200% of the natural global nitrogen load (13). Globally, inorganic fertilizer production accounts for approximately half of the excess anthropogenic fixed nitrogen (14). Other sources include organic fertilizers, sewage (through septic systems, spreading sludge on fields and old or leaking waste disposal sites), animal husbandry, cultivated leguminous plants, and fossil fuel combustion (primarily industrial and vehicular) (13,14).

Most ammonium (NH_4) in the environment is bound tightly to clay soils, while nitrate is soluble in water and has a great capacity to leach into groundwater (14,15). In North America, intensive agriculture is the primary source of groundwater nitrate contamination (14). The degree of contamination depends of the intensity and type of agriculture, as well as the permeability of the underlying aquifer (16). Following agriculture, leaking or

inadequate septic and sewage systems are the most commonly cited sources of groundwater nitrate contamination (16).

Nitrate levels in urban public water supplies are usually far below the MAC of 45 mg/L total nitrates, or 10 mg/L nitrate-nitrogen (15). However, recent surveys have shown patterns of highly elevated groundwater nitrate levels in many parts of Canada. While total nitrate concentrations up to 3 mg/L may occur naturally in some watersheds, higher concentrations are generally attributed to anthropogenic influences (17) Total nitrate concentrations up to 467 mg/L have been reported in Ontario and up to 1063 mg/L in Manitoba (15,18). A 1990 study of the Fraser Valley in British Columbia determined that over 60% of sampled wells had total nitrate concentrations exceeding the MAC.

In 1989, 13% of wells sampled in the study area within the Annapolis Valley had nitrogen-nitrate levels above the acceptable concentration of 10 mg/L, despite background levels of less than 1 mg/L (10). In 1999, a series of repeated nitrate measurements from 140 wells in the same study area showed nitrate-nitrogen concentrations above 5 mg/L in 43.65% of wells and concentrations above 10 mg/L in 19.41% of wells (11).

In Canada and the United States private water supplies are not usually subject to treatment or public health and environmental guidelines (17,19). Most rural families depend on groundwater accessed through private wells for consumption, despite elevated nitrate levels in groundwater relative to surface water (15). In Nova Scotia, over half of

the population acquires drinking-water from a groundwater source, including 90% of residents of the study area within the Annapolis Valley (10,20).

1.2.4 Spatial Variability of Groundwater Nitrate

A recent study examined the spatial association between land-use practices and groundwater nitrate in Prince Edward Island (PEI). Increased percentages of potato, grain and hay in watersheds were associated with increases in groundwater nitrate (21). A study in a farming community in Quebec also found a significant positive association between potato farming and increases in groundwater nitrate (22). In Ontario, cropping practices did not affect groundwater nitrate, though manure spreading led to an increased frequency of groundwater nitrate levels above the MAC and woodlot areas showed a decreased frequency of groundwater nitrate levels above the MAC (23). Similarly, a study on Nantucket Island found that forest and undeveloped land were negatively associated with groundwater nitrate (24). Two American studies found that residential density was associated with groundwater nitrate (24,25). A study in rural Washington showed a significant association between groundwater nitrate and surficial geology, though no association has been found between groundwater nitrate and surficial geology in Nova Scotia (10,25).

There is some evidence to suggest that well characteristics contribute to differences in drinking-water nitrate concentrations between wells. A 1995 study in Nova Scotia attributed some differences in nitrate concentrations between wells to well type (dug versus drilled) and well depth (10). However, no differences in nitrate concentrations

were associated with well type in a later study in the same area. Differences in nitrate concentration were related to well depth, but this association was not maintained when only drilled wells were included in the analysis (26). An examination of changes in nitrate concentration in wells according to sampling depth within wells showed that the highest nitrate concentrations occur 3 m below the water table, and that nitrate concentrations dissipated by approximately 67% at 6.5 m below the water table (23).

1.2.5 Temporal Variability of Groundwater Nitrate

Long-term changes in land-use practices have been linked to trends in groundwater nitrate concentrations in Canada and the United States (27,28). Long-term changes in land-use traits that have been linked with increases in groundwater nitrate include: expanding urban areas, more intensive agricultural practices and the growth of un-sewered communities bordering metropolitan areas (28).

However, there is mixed evidence of the existence of long-term trends in groundwater nitrate concentration in North America. An examination of historical records in Iowa found no significant changes in nitrate concentrations in groundwater between 1982 and 1995 (29). Similarly, a study in Prince Edward Island found no significant annual trend in groundwater nitrate over a 16-year period (30). However, a long-term study in the midwestern United States identified increases in nitrate concentrations, though exclusively in shallow wells (31). Three North American studies have shown significant long-term increases in groundwater nitrate concentrations (15,32,33).

There are also mixed-reports on the presence of monthly and seasonal trends in groundwater nitrate concentrations. In PEI, groundwater nitrate concentration was significantly associated with month of sampling. Though season showed no independent effect, an interaction term between season and land-use was significant (27). Significant differences in groundwater nitrate between months, but no seasonal pattern, was shown in the Annapolis Valley (26). However, other North American studies found no significant independent monthly or seasonal effects on groundwater nitrate (23,28,34).

The same study in PEI suggested that there is greater variation in nitrate concentrations between wells than within wells. It was found that 92% of variation in nitrate could be attributed to differences between sites while only 0.04% was attributed to differences between years (30). This pattern is supported by a recent study that sought to determine if groundwater nitrate levels are sufficiently stable to conduct epidemiological studies using historical water quality data (35). A total of 853 private wells in the midwestern United States were assigned to one of four exposure categories (background, low, moderate and high) based on water samples taken in the summer of 1994. One year later, the majority of the wells fell into the same category (75.4%). When the exposure category of wells changed, most wells shifted to the lower adjacent exposure category (ie. moderate to low)(35). Ruckart *et al.* (2008) (35) concluded that the: "results are somewhat reassuring for the design of epidemiologic studies."

1.2.6 Nitrates and Human Health

Ingested nitrate may be converted to nitrite by microbial reduction in saliva, or in the stomach during instances of increased pH or infections with diarrhoea-producing bacteria (15,36). Evidence has also shown that nitrites can react with amines and other nitrosatable compounds to produce highly reactive N-nitroso compounds in the stomach (15,36). This reaction is facilitated by bacterial catalysts (36). The carcinogenicity of N-nitroso compounds has been demonstrated in animal models, and human epidemiologic studies have established that N-nitroso compounds and nitrites likely play a role in gastric cancer development (37-42). Studies have shown positive associations between nitrate and other cancers, hypertension, diabetes, methemoglobinemia and a variety of adverse birth outcomes, including intrauterine growth restriction, spontaneous abortion, prematurity and congenital anomalies (41,43-48).

1.2.7 Nitrates and Congenital Anomalies

Several animal studies have shown that nitrates and other nitrogenous compounds can cross the placenta and affect the developing fetus during pregnancy (36,49). N-nitroso compounds have demonstrated teratogenicity and have been shown to induce congenital anomalies in several animal models (37-40,50). The teratogenic potentials of nitrate and nitrite have not been clearly established, though animal studies have shown that the injection of extremely high concentrations of nitrate during pregnancy can produce anomalies in mammals (50). In humans, the critical window in which nitrogenous compounds could produce congenital anomalies has been shown to be between the 2^{nd} and 10^{th} weeks of gestation (51).

An association between nitrogenous compounds and congenital anomalies among humans was first proposed in 1972, following a retrospective descriptive study undertaken in the United Kingdom (4). A significant correlation was found between the per capita consumption of nitrate and nitrite-cured meats and anencephaly, for both place and time (4). The methodology of that study was not sufficiently rigorous to establish a clear relationship between nitrogenous compounds and congenital anomalies, though it served to pique further interest in the influence of nitrates on the incidence of congenital anomalies.

Most published studies on congenital anomalies and drinking-water nitrate have shown positive associations and a few studies have shown no association. A Swedish study compared the average local drinking-water nitrate concentrations for infants born with NTD to those born without a congenital anomaly, and found no significant difference (52). In 2007, an American study found that there was no significant correlation between monthly birth rates of infants with abdominal wall defect and state-wide surface water nitrate levels (53). However, a visual examination of plots of the monthly birth rates of infants with abdominal wall defects and monthly nitrate levels at the time of conception suggested that the two shared similar patterns of increase and decrease (53).

A number of studies have shown positive, albeit often non-statistically significant, associations between drinking-water nitrate and congenital anomalies. A 1984 study by Dorsch *et al.* (3) in South Australia was the first to document a positive association

between drinking-water nitrate levels and incidence of congenital anomalies. Women who consumed water with total nitrate concentrations between 5 mg/L and 15 mg/L were 2.6 times more likely than women who consumed water with total nitrate concentrations below 5 mg/L to give birth to a child with a congenital anomaly (RR=2.6, 95% CI =1.6-4.1); those who consumed water with total nitrate concentrations above 15 mg/L experienced a risk 4.1 times greater (RR=4.1, 95% CI= 1.3-13.1) (3). A study in New Brunswick by Arbuckle *et al.* (5) found a non-significant dose-response relationship between prenatal exposure to nitrate in drinking-water and incidences of central nervous system (CNS) anomalies. When well-water was considered in isolation, total nitrate concentrations of 26 mg/L showed a moderate increase in risk of CNS anomalies (ROR=2.3; 95% CI = 0.73-7.29) (5). More recently, a California case-control study found a progressively increased risk of anencephaly according to higher levels of total nitrate exposure for groundwater drinkers only (OR=2.1, 95% CI = 1.1-4.0 for exposure concentrations of 5-15 mg/L; OR=2.3; 95% CI = 1.1-4.5 for exposure concentrations of 16-35mg/L; OR=6.9; 95% CI = 1.9-24.9 for exposure concentrations of 36-67mg/L.) (6). A Swedish study found that infants exposed *in utero* to more than 2 mg/L nitrate-nitrogen had a non-significantly elevated risk of cardiac defects (OR = 1.18, 95% CI = 0.97-1.44) (7). A study investigating the effects of dietary nitrates and nitrite, and nitrosatable drugs, on NTD among Mexican-American women found that women exposed to nitrate concentrations in excess of 3.5 mg/L had a non-significant increased risk of delivering a child with a NTD (OR=1.9, 95% CI=0.8-4.6) (54).

1.2.8 Limitations in Existing Research on Nitrate and Congenital Anomalies

The studies presented above are limited by methodological weaknesses that make it difficult to infer a concrete relationship between the exposures and outcomes of interest. One literature review suggested that the claim of a strong association between groundwater nitrates and the risk of congenital anomalies by Dorsch *et al.* (3) was premature due to a weak study design (50). Poor estimation of nitrate exposure levels and an association across a broad array of anomalies were given as evidence of a non-causal relationship (50). While the studies by Arbuckle *et al.*(5), Brender *et al.* (54) and Croen *et al.* (6) identified a more specific relationship between CNS anomalies and nitrate exposure, two of the authors identified the potential for misclassification of nitrate exposure levels, residual confounding due to uncontrolled and unidentified risk-factors and low power following stratification by type of congenital anomaly. A review supported the conservative interpretation of the study results by Arbuckle *et al.* (5,50)

Most previous studies on the association between incidence of congenital anomalies and prenatal drinking-water nitrate have ascertained cases and controls from birth registries or congenital anomaly monitoring programs (5-7,52,53). One study enrolled livebirths as from three hospitals as cases and controls over a set time period (3). Another study, part of a broader research program, actively ascertained cases with NTD from genetic clinics, ultrasound centres, hospitals, birthing centres, prenatal clinics and midwives (54). Prenatally diagnosed fetuses with NTD were included in this study, as well in the study by Croen *et al.* (6,54). Therefore, a significant limitation among other studies is their

inability to account for second trimester terminations of pregnancy for prenatally diagnosed congenital anomalies, especially those other than NTD.

Nitrate exposure assessments in the studies by Arbuckle *et al.* (5), Brender *et al.* (54), and Cedergren *et al.* (7) were designed to be accurate and precise. Arbuckle *et al.* (5) and Brender *et al.* (54) took water samples from indoor taps shortly after birth, while Cedergren *et al.* (7) used GIS and information from local water suppliers to determine the level of nitrate exposure of those living in municipalities, though private wells could not be assessed. The studies by Mattix *et al.* (53), Dorsch *et al.* (3) and Croen *et al.* (6) were limited by potential misclassification of prenatal nitrate exposure. The study by Mattix *et al.* (53) had the greatest risk of misclassification, as they used broad state-wide comparisons of surface water nitrate, rather than attempting localized estimates of drinking-water nitrate. Croen *et al.* (6) described a process in which water companies measured nitrate values every three years, and the most recent measurement taken nearest to the home was used to estimate exposure during pregnancy. However, in some cases the average nitrate exposure level for all homes was imputed, introducing misclassification of nitrate exposure levels (6). Dorsch *et al.* (3) asked families to recall the identity of their water provider during the pregnancy period and whether the source was groundwater, lake water or rain water (3). This process may have introduced recall bias if the parents of children with congenital anomalies had considered their environment during pregnancy differently than other parents.

The impact of poor nitrate measurement on the results of the previous studies remains unclear. The potential level of misclassification in the study by Mattix *et al.* (53) may have masked a positive or negative association. The potential for recall bias in the study by Dorsch *et al.* (3) may have exaggerated the association between nitrates and congenital anomalies. Imputing average nitrate exposure for missing values in the study by Croen *et al.* (6) may have introduced non-differential misclassification of nitrate exposure, attenuating any relationship between the exposure and outcome.

Dorsch *et al.* (3), Arbuckle *et al.* (5) and Erickson *et al.* (55) used matching to reduce the likelihood of confounding by certain risk factors. Most studies also used some type of statistical adjustment to control for covariates.

Dorsch *et al.* (3), Arbuckle *et al.* (5), Croen *et al.* (7) and Brender *et al.* (54), used interviews after birth to obtain information on potential confounders, introducing the potential for recall bias. Dorsch *et al.* (3) examined the following potential confounders: infant sex, maternal marital status, nationality, water source, area of residence and paternal occupation. Only infant sex, area of residence and water supply influenced the association between congenital anomalies and drinking-water nitrate. Croen *et al.* (6) used statistical adjustment to control for race, maternal age, maternal income, maternal body mass index, maternal vitamin use and dietary nitrate exposure, though none had a substantial effect on the crude odds ratios. Arbuckle *et al.* (5) considered potential confounding by maternal age, infant birth order, water source, chloride level in drinking-water and maternal birthplace. Water source, chloride and maternal birth place influenced

the association between congenital anomalies and drinking-water nitrate and were retained in the final model. Cedergren *et al.* (7) used statistical techniques to adjust for maternal age, parity, smoking, education and trihalomethane exposure, though adjustment did not influence the odds ratio. Brender *et al.* ascertained periconceptional dietary intakes of nitrate and nitrate from food frequency questionnaires, though the frequency and amount of water consumed was not measured. Brender *et al.* also examined the effects of education, income, body mass index (BMI) , folate intake, supplement use, caloric intake, dietary vitamin C intake, smoking, dietary nitrosamine intake, nitrosatable drug intake, serum B_{12}, hyperinsulinemia, fever, solvent exposure and stressful life events, though only household income BMI and folate intake were included in the final logistic regression models. Maternal education, fever and nitrosatable drug exposure during the periconceptional period influenced the relationship between congenital anomalies and drinking-water nitrate.

Maternal folate intake, maternal medication-use and disease status have not been considered in many of the studies. Residual confounding due to unacknowledged potential confounding variables may have attenuated the relationship between prenatal nitrate exposure and congenital anomalies in these studies. There are a number of potential risk factors for congenital anomalies that were not considered in any of the studies described above, Certain infectious agents, such as rubella and varicella may contribute to the development of congenital anomalies if transmitted to the fetus (1). Many drugs, such as thalidomide, anticonvulsants, folic acid antagonists and retinoids, also have known teratogenic effects (1). Exposure to alcohol may also influence the

development of congenital anomalies, though the effects of smoking remain under debate (1,56-60). Low folic acid intake, less than 400 μg per day during early pregnancy, increases the risk of neural tube defects, though this can be countered by folic acid supplementation and fortification (12,61). Maternal health characteristics such as obesity and diabetes are established risk factors for congenital anomalies (1,62-69). Though the evidence is less clear, primiparity, and thyroid disease have also been listed as potential risk factors for congenital anomalies (1,70-73). Many studies have observed associations between increased maternal age and increased incidence of congenital anomalies, while some have also observed associations between young maternal age with increased incidence of congenital anomalies (1,57,74,75). It is plausible that some of these potential risk factors for congenital anomalies could be related to nitrate exposure, and thus confound the relationship between prenatal nitrate exposure and congenital anomalies.

1.2.9 Exposure Assessment in Research on Drinking-Water and Pregnancy Outcomes

Establishing appropriate exposure assessments is one of the greatest challenges in environmental epidemiology. Adverse health outcomes typically become apparent many years after environmental exposures, making it difficult to reconstruct an accurate exposure history. Exposure misclassification is the greatest source of bias in studies examining drinking-water contamination and adverse pregnancy outcomes (76). With regards to congenital anomalies, even through the latent period between exposure and diagnosis is short, the outcome is sufficiently rare to make it difficult to conduct prospective studies. Though drinking-water data in municipalities are carefully recorded, samples are usually taken at only at a small selection of points along the distribution

system a few times a year (76). Testing of private wells is the responsibility of well owners, and no public records of the water quality of private wells are maintained (17,18,77). Therefore, researchers must reasonably extrapolate exposure over both space and time (76).

Previous studies examining the association between congenital anomalies and drinking-water nitrate have used various methods to ascertain exposure status. One study asked families to recall their water source during pregnancy, and used historical data to reconstruct nitrate levels in that water source (3). Most studies linked the address of each study participant to the nearest municipal water supply (6,7,53). A few studies used actual nitrate measurements taken from the home tap shortly after birth, one of which also used interviews and questionnaires to assess nitrate exposure via food and drug ingestion (5,54).

Studies examining the association between congenital anomalies and exposure to other drinking-water contaminants have used similar methods for ascertaining exposure status. Several studies have used the mother's address at the time of delivery to estimate drinking-water contaminant exposure during pregnancy from municipal records (78-80). Changes in individuals' residential history, variation in actual sources of drinking-water, as well as variation in the consumption of drinking-water and other means of exposure further complicate exposure assessment (76). Three studies have interviewed mothers to determine their addresses during the first trimester of pregnancy, and used municipal records from that time to estimate exposure to drinking-water contaminants (44,81,82).

One of these studies also used the interview to ascertain information on water consumption habits (82). Only one known study was identified in which the investigators prospectively interviewed pregnant women regarding their drinking-water consumption habits prior to birth (83).

Several studies examining drinking-water nitrate exposure and non pregnancy-related health outcomes attempted to overcome these limitations by establishing approximate lifetime drinking-water exposure estimates based on historical municipal drinking-water nitrate records for all addresses at which study participants had lived (84,85). A few of these studies also included data from questionnaires on eating habits and drug use (84,86). While studies that collect extensive histories from participants can theoretically provide a much more precise exposure assessment, they are limited by the potential for recall bias (87).

1.2.10 Exposure Assessment Using GIS

Existing techniques for assessing exposure to drinking-water contaminants, including nitrate, during pregnancy are sufficient to enable research in municipal areas. A workgroup report prepared following a symposium on drinking-water nitrate and health at the 2004 meeting of the International Society for Environmental Epidemiology emphasized the need to study users of private wells (8), which was reinforced by a review paper (9). Furthermore, this workgroup report asserted that GIS is a promising approach for better estimations of nitrate exposure risk (8).

Simple GIS techniques can be used to assess rates of disease based on proximity to a hazard, to map disease prevalence, to undertake surveillance, or to identify study population (88,89). Geographic modelling uses GIS to create new or modified variables to enable more precise exposure estimates, often at the individual level, and is particularly useful when contaminant measurements are limited, such as in the investigation of drinking-water contamination in rural areas (88). Geographic modelling has been used in only a handful of epidemiological studies involving groundwater contamination (90,91).

A number of geographical modelling techniques exist that can be used to estimate groundwater nitrate concentrations at all points in an area, based on measurements from a sample of points. These include spatial methods such as "zones of contribution", vulnerability rating models, process-based methods, and geostatistical methods (91,92).

Various spatial methods have been used to estimate nitrate exposure. A study in Quebec compared well locations to nitrate sources, such as potato fields, to assess the likelihood of drinking-water contamination and the need for a public health intervention (22). In Cape Cod, zones of contribution were drawn around municipal and private wells. Land use data and nitrate concentrations from wells were combined to estimate drinking-water nitrate exposure within each zone of contribution (91).

The most commonly used vulnerability rating model, the DRASTIC model, develops a rating of the risk of contamination based on depth to water table (D), aquifer recharge

(R), aquifer media (A), soil media (S), topology or slope (T), impact of the vadose zone (I), and conductivity (C) (93). The DRASTIC model has been criticized for using only a narrow range of parameters and for fostering subjectivity in the ratings assigned for each parameter (92).

Process-based models encompass information on water movement and the fate and transport of contaminants. These models are generally very complex and require large amounts of data, and are therefore usually reserved for large projects (88,92).

Geostatistics is a branch of statistical modelling originally created for use in the mining industry that is adapted to estimating the spatial variability of quantities based on existing data (94). The use of geostatistical methods is appropriate when few field observations are available, or when understanding of the processes of fate and transport within the system of interest is incomplete (94). Geostatistical method have previously been used to assess the relationship between groundwater characteristics and acute myocardial infarction (95).

CHAPTER 2: HYPOTHESIS

The null hypothesis tested in this project is that there is no association between the incidence of congenital anomalies and elevated prenatal drinking-water nitrate exposure level after controlling for covariates, including: sex of infant, month of conception, year of conception, maternal demographic traits, maternal risk factors and other water quality variables. The alternative hypothesis is that there is an association between the incidence of congenital anomalies and elevated prenatal drinking-water nitrate exposure level after controlling for covariates. Two sets of controls were used in this study in order to examine community level effects on the associations that were assessed.

The Drinking-water nitrate exposure levels used in statistical analyses are based upon estimates of the nitrate concentration of the tap-water in the homes of all study participants. All nitrate concentrations in this study are reported in mg/L nitrate-nitrogen, unless otherwise noted.

CHAPTER 3: METHODOLOGY

3.1 Study Design

A case-control design, including births from 1988 to 2006 and two distinct groups of controls was used in this study. All study participants were selected from either the Nova Scotia Atlee Perinatal Database (NSAPD) or the Fetal Anomaly Database (FADB). Participant selection and data compilation were done by the Reproductive Care Program of Nova Scotia (RCP). All infants born to mothers living in the Annapolis Valley study area and diagnosed with a major congenital anomaly according to the NSAPD were included as cases. All fetuses diagnosed with a congenital anomaly that did not survive to 20 weeks or were electively terminated according to the FADB were also included as cases. One group of controls included infants without a major congenital anomaly from the NSAPD born to mothers residing in the Annapolis Valley study area at the time of delivery. The other group of controls included infants without a major congenital anomaly from the NSAPD born to mothers residing in the Halifax Regional Municipality (HRM) at the time of delivery.

Cases and controls were individually matched at a 1:3 ratio based on sex and date of conception. For each case, six controls were randomly selected; three from the Annapolis Valley study area and three from HRM. Controls were of the same sex as cases, and the dates of conception of cases and controls differed by no more than 30 days. Cases and controls were matched on sex because previous studies have suggested that the male fetus could be more vulnerable to malformation than the female fetus, and that the incidence of congenital anomalies among males is higher than among females (74). This difference

may be, in part, attributable to the inclusion in research of anomalies affecting almost exclusively males (74).

Cases and controls were matched on date of conception because of existing evidence that suggests that there is temporal variation in drinking-water nitrate. Nitrate concentrations available in rural areas within the Annapolis Valley study area were collected only over 8 months, despite the births of study participants spanning 18 years. In the event that there was non-random temporal variation in nitrate concentrations over the study period, the individual matching procedure prevented differential selection of cases and controls from periods with high or low nitrate concentrations.

3.1.1 Inclusion Criteria

Infants meeting the following criteria were eligible to be enrolled in the study.

1) Born to a mother living in HRM or in the Annapolis Valley study area between January 1, 1998 and December 31, 2006
2) Registered in either the NSAPD or the FADB
3) The address of the mother at the time of delivery was available in the NSAPD, or could be retrieved from health records held at the IWK health centre, and could be linked to a latitude and longitude

3.1.2 Exclusion Criteria

Multiple births were not included in the study because there are congenital anomalies that are specific to multiple births and cannot occur among singleton births.

3.2 Study Area

The Annapolis Valley is located in south-western Nova Scotia, a province in eastern Canada. The Annapolis Valley is delineated by the North Mountain and the South Mountain and is contained in Annapolis County and Kings County. Annapolis County and Kings County are bounded by the New Minas Basin and the Bay of Fundy to the north. The valley floor consists of a rolling topography, underlain with Wolfville formation sandstone and Blomidon formation shale. Water movement in the Wolfville formation occurs through intergranular pore spaces (96). Wells drilled into the Wolfville formation typically yield 450-2300 L/min (96). In the Blomidon formation, few intergranular pores exist in the fine-grained rocks and water movement is limited to joints (96). Well yields in the Blomidon formation are rarely above 14 L/min (96). Water quality in the Annapolis Valley is usually good, though it is susceptible to contamination from surficial sources (10,96).

The general direction of groundwater flow in the Annapolis – Cornwallis Valley is down from the mountain tops into the centre of the valley (97). Groundwater in the Annapolis Valley flows approximately in a northeasterly direction; most of it travels beneath the Annapolis Valley floor and into the New Minas Basin and the Bay of Fundy (97). The water table is the deepest below the ground surface along the North and South Mountains and the closest to the ground surface along the valley floor (97). Groundwater beneath the North Mountain and South Mountain generally has low or very low vulnerability to

surface contamination, while groundwater beneath the valley floor has moderate or moderately high vulnerability to surface contamination (97).

Several municipal water supplies are encompassed in the study area. They are: the Municipality of the County of Kings, Canning, Wolfville, Kentville, New Minas and Port Williams. The Municipality of the County of Kings water commission provides water to approximately 550 customers in Greenwood, Aylesford and Sandy Court (98). Water is supplied from two groundwater wells and disinfected via chlorination (98). The town of Wolfville also accesses water from two groundwater wells (99). The town of Kentville accesses water from one surface water source; disinfection is by chlorination (100). The town of Port Williams Water Commission serves approximately 930 customers (101). Water is drawn from four wells and disinfected by chlorination (101).

The Halifax Regional Municipality (HRM) is located on the Nova Scotia Atlantic coast and includes the municipalities of Halifax, Dartmouth, Bedford and Sackville, as wells as a number of smaller towns communities and rural areas. Only individuals in HRM served by the two main water supply plants of the Halifax Regional Water Commission, the J. D. Kline Water Supply Plant and the Lake Major Water Supply Plant, were included in this study. These water supply plants serve the municipalities of Halifax, Bedford, Sackville, Dartmouth, Timberlea, Fall River, Waverly, Cole Harbour, Westphal and Eastern Passage. (102). Both plants supply residents with filtered surface water that is disinfected using chlorination ((102).

3.3 Study Population

Based on the 2006 Canada Census, the study area within the Annapolis Valley has a population of 60,035 (103). Approximately 48% of the population is male and 52% of the population is female (103). The median age of the population is 41.7 years and 83% of the population is age 15 or older (103). Approximately 62% of the population aged 15 or older worked for pay in 2006, and in 2001 census the median total income of all persons over 15 years was $17,592.00 (103,104). Most of the Annapolis Valley study area is classified as a moderate Metropolitan Influence Zone (MIZ), defined as a region in which 5-30% of those employed commute to a Census Metropolitan Area (CMA) (103,105). The municipality of Kentville is the only CMA in the Annapolis Valley study area (103). Statistics Canada defines a Census Metropolitan Area as one or more adjacent communities, highly integrated based on commuter flow, centred around an urban core with a population of at least 100,000 (105).

According to the 2006 census, HRM has a population of 372,679 (103). Approximately 48% of the population is male and 52% of the population is female (103). The median age of residents of HRM is 39 years, and 83% of the population is age 15 or older (103). Approximately 69% of the population aged 15 years or older worked for pay in 2006, and the median income of all persons over 15 years was $22,986.00 according to the 2001 census (105,106). All of HRM is considered a CMA (103).

3.4 Health Data Sources

All study participants were selected from the Nova Scotia Atlee Perinatal Database (NSAPD) and the Fetal Anomaly Database (FADB). The NSAPD is one of only three population-based perinatal health databases in Canada (1). It was established in 1988 and is managed by the Reproductive Care Program of Nova Scotia (RCP). The NSAPD contains information on maternal and infant demographics, as well as information on medical procedures, interventions, diagnoses (including congenital anomalies) and health outcomes for all registered births in Nova Scotia (1). The NSAPD captures information on all births that took place after at least 20 weeks of gestation, or for which the infant weighed at least 500g. Data are obtained from hospital records, physician reports, prenatal diagnostic facilities, cytogenetic laboratories, maternal serum screening programs and vital statistics (1). All congenital anomalies are listed by both type and related syndrome. Re-abstraction studies indicated that the data collected is reliable and of good quality (106,107).

The FADB is a population-based congenital anomaly database. It was established in 1992 and is managed by the Division of Maternal-Fetal Medicine in the Department of Obstetrics at the IWK Health Centre (1). The FADB records information on all fetal anomalies diagnosed during pregnancy, including fetuses diagnosed with congenital anomalies that did not survive to 20 weeks of gestation and second trimester terminations for fetal anomalies, for women referred from Nova Scotia, New Brunswick and Prince Edward Island (1). Data are obtained in the same fashions as for the NSAPD (1).

3.5 Water Quality and Nitrate Data Sources

Drinking-water nitrate concentrations in rural areas were estimated from the nitrate concentrations of samples from a series of wells monitored by the Nova Scotia Department of Environment and Labour and the Nova Scotia Agricultural College (1,10). Nitrate concentrations from approximately 240 private wells in the Annapolis Valley study area were measured in the summer of 1989 (10). During the process to select wells for sampling, those wells with a higher risk of contamination according to a modified DRASTIC model were given a higher weight of selection (10). This resulted in a higher concentration of sampling locations falling in the regions of the Annapolis Valley more susceptible to surficial contamination, primarily along the valley floor (10). Efforts to contact initial study participants succeeded in contacting the owners of only 140 wells for follow-up sampling from 1999 to 2000 (11,26). Data used for analysis in rural areas for this project were from the 140 wells sampled from July 1999 to February 2000.

For all municipalities in this study, including HRM, nitrate concentrations were provided by the Nova Scotia Department of Environment and Labour as well as individual municipal water commissions. Water samples were taken at irregular intervals over the study period; roughly every 2 years. Each municipal water supply manager provided either a list of addresses served by the distribution system, or a map of the distribution system, to assign drinking-water sources to study participants.

3.6 Data Analysis

The data analysis for this project consisted of four major tasks: 1) data validation and descriptive analysis for health variables; 2) data validation and descriptive analysis for water variables; 3) creating a map to geographically represent drinking-water nitrate exposure in the study area; and 4) modelling the association between congenital anomalies and drinking-water nitrate exposure level, considering other covariates. Data analyses were done using SAS Institute SAS ® v. 8 and ESRI ArcGIS ™ .

3.6.1 Variables Included in the Study

The variables included in the logistic regression models developed for this project can be divided into four categories: 1) matching variables; 2) maternal demographic variables; 3) maternal risk factors and; 4) water quality variables and nitrate.

The matching variables were sex of infant and date of conception, calculated based on the infant's date of birth and the best estimate of gestational age contained in the NSAPD or the FADB. Cases and controls were matched such that the dates of conception of the controls fell within 30 days of the dates of conception of the cases. For data analysis, date of conception was categorized as season of conception and year of conception. The demographic variables included in the study were: maternal age at conception and maternal parity, defined as the number of times a woman has given birth to an infant greater than 20 weeks gestation. The maternal risk factors included in the study were: smoker (mother smoked either pre-pregnancy or at first prenatal visit), pre-existing or gestational diabetes, pre-existing thyroid disease, patient-reported folate supplementation,

patient-reported pre-pregnancy weight, and conception before or after folate fortification in Canada (determined by year of conception). The water quality variables included in the study were surface or ground water source, municipal or private water supply, and nitrate exposure level. All water quality variables and nitrate concentration estimates were established based on the latitude and longitude of the mother's address at the time of delivery.

3.6.2 Data Analysis of Health Variables

The frequencies of diagnoses of congenital anomalies by body system (as defined by RCP) were determined for the entire study period, as well as for the period prior to the widespread fortification of food with folic acid in Canada (1987-1997), and the period following folic acid fortification (1988-2006). Frequency tables were created for all covariates included in the study. Most covariates were coded as categorical variables by RCP, with the exception of maternal age at conception, pre-pregnancy weight and nitrate concentration. The continuous variables were amalgamated into categories prior to the creation of frequency tables. The categories of nitrate concentrations were termed drinking-water nitrate exposure levels.

3.6.3 Descriptive Statistics for Water Quality and Nitrate Data

The water quality data were analyzed separately in three different groups: municipal water supplies in the Annapolis Valley, municipal water supplies in HRM, and rural private wells in the Annapolis Valley study area. Often, multiple samples were taken from the public water supply systems on the same date, usually from different points

within the distribution system. Multiple measurements on a single day were replaced with a single value, the median of all measurements taken on that date.

For each group, descriptive statistics were performed and the distribution of the data was assessed for normality using visual observations and the Kolmogorov-Smirnov goodness-of-fit test. Data were then transformed using various techniques, and the transformed data were assessed for normality using the techniques described above. The data that were the closest to normally distributed following transformation were used in the analysis, and back-transformed for interpretation.

To determine spatial and temporal effects on nitrate concentrations, linear mixed effects models were used to assess the effects of location of sample, month of sample and year of sample on nitrate concentrations. The variables representing month and year were analyzed categorically, as differences between months and years were sought, rather than evidence of a linear trend. Location of sample and year were considered random effects, while month of sample and year of sample were considered fixed effects. This structure accounted for clustering of months within years and years within locations. Similar methods have previously been used to assess temporal changes in groundwater nitrate measurements (21,35).

3.6.4 Exposure Assessment

The exposure assessment for this project was conducted using ESRI ArcGIS ™. The exposure assessment consisted of four main steps: 1) interpolating groundwater nitrate

exposure levels in rural areas through a geostatistical model; 2) delineating the boundaries of municipal water supplies and calculating the median nitrate exposure levels for these municipalities; 3) combining the rural interpolation with the municipal measurements and boundaries to create a single exposure map of the entire study area; and 4) using the Nova Scotia Civic Address file to link the addresses of study participants to a drinking-water nitrate exposure level from the exposure map.

3.6.5 Interpolating Nitrate Concentrations in Rural Areas

The "Geostatistical Analyst" tool in ArcGIS™ was used to interpolate groundwater nitrate measurements in rural areas. Prior to data analysis, it was not known what type of geostatistical analysis would yield the best interpolation. Therefore, interpolation models were created using both Inverse Distance Weighting (IDW) and kriging. Descriptions of these methodologies are based on a textbook by Kitandis (94).

Inverse distance weighting estimates an unknown value at a particular point by determining the distance-weighted average of the measured values within a neighbourhood, normally specified as the "n" closest points to point at which the value is unknown. Several different interpolations were generated using IDW, each with a different neighbourhood.

Kriging determines the "best linear unbiased estimator" using stochastic processes which are described by a variogram function. A linear estimator is a linear equation that estimates the value of an unknown based on the probability that the unknown is equal to

the values of known measurements; in kriging this equation is then used to estimate the values at all points for which they are unknown. The "best" linear estimator has the lowest mean square error. A linear estimator is "unbiased" when value of the estimation error is equal to zero. A variogram function is a smooth line that passes through a scatter plot that is generated by plotting the separation distances on the x-axis and the square difference between measurements on the y-axis, for all measurement pairs. To select an appropriate variagram function for kriging, an empirical variogram is plotted using all measurements and a model variogram is fit to the empirical variogram. The adjusted model variogram and a system of linear equations are used to generate an interpolation through kriging. Ordinary kriging, which assumes that there is a constant, albeit unknown, trend in the measurements, was used in this project. A number of different interpolations were generated using ordinary kriging. Some interpolations were generated using a spherical variogram, while others were generated using an exponential variogram. Each interpolation had a different neighbourhood.

In the best interpolations, the mean error and mean standardized error close to zero, the root mean square standardized error close to one, and the root mean square error is close to the average standard error. The interpolation that had the mean error nearest to zero, and was close to meeting the other criteria, was selected for use in the remaining analyses and was labelled the primary nitrate interpolation. This interpolation was validated by re-creating it using only 90% of the data points, and comparing it to the original interpolation. To assess face validity, the selected interpolation was also compared to a

DRASTIC model of groundwater nitrate in the same study area by Atari (11) and nitrate concentrations presented in a Hydrogeological Atlas of the Annapolis Valley (97).

3.6.6 Delineating Municipal Water Distribution Systems

The lists of addresses and maps provided by municipal water supply managers were used to draw freehand polygons representing the geographic range of each water supply distribution system in ArcGIS™. A map of each community, with labelled roads and the highlighted polygon delineating the distribution system was sent to each manager. Water supply managers advised changes to the polygons. This process was repeated until all water supply managers verified that the polygons adequately described the geographic ranges of the water supply distribution system.

3.6.7 Combining Rural and Municipal Nitrate Concentration Maps

The map representing rural groundwater nitrate concentrations in the study area derived from interpolation and the map representing the drinking-water nitrate concentrations in the municipal polygons were combined using ArcGIS™. All geographic points in the study area were associated with a single nitrate concentration, water source (ground or surface) and a denotation of whether or not it was served by a municipal water supply (yes or no). This generated a raster file estimating groundwater nitrate concentrations, water source and municipal water supply on a grid throughout the Annapolis Valley study area.

3.6.8 Linking Addresses to Water Quality Information

A spreadsheet listing the latitude and longitude of the home of each study participant at the time of delivery was provided by RCP. Using ArcGIS™, all data points that did not fall within the Annapolis Valley study area or HRM were deleted.

As described above, a raster file estimating groundwater nitrate levels on a grid throughout the Annapolis Valley study area was developed. Polygons outlining geographic regions with the same drinking-water nitrate estimates were generated. These polygons were overlain with address data points and the two datasets were joined such that each address point was associated with the nitrate concentration polygon beneath it. The same procedure was used to assess which data points represented homes served by municipal water distribution systems, and which data points represented homes receiving drinking-water from surface and ground water sources. A spreadsheet associating the latitude and longitude of the home of each case and control from the Annapolis Valley study area with a drinking-water nitrate concentration estimate, municipal water distribution and water source was generated.

ArcGIS™ was also used to identify all study participants that were born to a mother living in HRM. A spreadsheet was generated that associated all study participants from HRM with a drinking-water nitrate concentration estimate, surface water source, and water from a municipal water supply based on data from the HRM water supply plants. The files pertaining to study participants from HRM and study participants from the

Annapolis Valley were combined. This file was returned to RCP, where the geographic identifying information was removed and the health information was inserted.

3.7 Statistical Modelling

Unconditional logistic regression models were used for all further analyses. Unconditional logistic regression is appropriate for use when study participants are matched on very few parameters relative to the total sample size, provided that the matching variables are included in the unconditional logistic regression model (108).

Unconditional logistic regression was used to determine the crude odds ratios of the relationships between incidence of congenital anomalies and each covariate. Further analysis was done using unconditional logistic regression models constructed in an additive fashion based on a theoretical framework. Covariates were added to the model in groups to assess their impact on the association between congenital anomalies and drinking-water nitrate exposure levels, which was assessed upon the inclusion of the matching variables in the model. Covariates were added to the model by group in the following order: 1) demographic variables, 2) maternal risk factors, 3) water quality variables and, 4) nitrate.

The analysis was initially conducted using only controls from the Annapolis Valley, and repeated using only controls from HRM. For models using controls from HRM, the models did not include water quality variables and drinking-water nitrate exposure level. Models were also generated after stratification of the study participants from the

Annapolis Valley by ground or surface water, rural or municipal residence, and conception before or after folic acid fortification.

3.8 Data Access and Ethical Review Process

Approval for access to data in the NSAPD was granted by the Joint Data Access Committee of the Perinatal Epidemiology Research Unit (PERU), the Population Health Research Unit (PHRU), and the Reproductive Care Program of Nova Scotia (RCP) in November 2006. Approval for the use of data in the FADB was granted by the Fetal Anomaly Database (FADB) Data Access Committee in May 2007. Both committees are affiliated with Dalhousie University and the IWK Health Centre. The project also received ethical approval from the IWK Health Centre Research Ethics Board in May 2007 and the Annapolis District Health Authority Research Ethics Board in November 2007.

CHAPTER 4: RESULTS

4.1 Participant Selection

A total of 678 cases with congenital anomalies were identified in the Annapolis Valley study area: 566 cases were identified in the NSAPD and 112 cases were identified in the FADB. All cases were appropriately selected from within the Annapolis Valley study area. Cases in the FADB had a prenatal diagnosis of congenital anomaly and underwent spontaneous pregnancy loss or second trimester termination. The addresses of all cases in the NSAPD could be linked to latitude and longitude. Of the 112 cases in the FADB, the addresses retrieved from patient records could be linked to a latitude and longitude for only 40. In most cases where addresses did not link to a latitude and longitude, the study participant was conceived prior to inception of the provincial civic address system in 1998. Only those cases and controls who had addresses that could be linked to latitude and longitude could be included in the study. Therefore, approximately 72 cases from the FADB conceived between 1992 and 1998 were excluded from the study because their addresses could not be linked to a latitude and longitude. A total of 606 cases could be linked to a latitude and longitude and were included in the study.

A total of 3479 controls were selected, though 543 had addresses that did not fall within the Annapolis Valley study area or the area of HRM served by the two main water treatment plants. The matching procedure used in this study selected controls based on county of residence or residence in HRM. Estimates of nitrate concentrations were available for the entire Annapolis Valley study area, but available only for select municipalities within HRM. Study participants selected from other municipalities within

HRM could not be included in the analysis and represented most of the deleted data points, resulting in fewer controls from HRM (n=1301) than from the Annapolis Valley study area (n=1635).

4.2 Results of Water Quality Data and Nitrate Data Analysis

4.2.1 Descriptive Statistics for Municipal and Rural Data

The nitrate concentrations from the municipal water supplies in the Annapolis Valley study area and the municipalities within HRM served by the two main water treatment plants are presented in Table 1. Nitrate concentrations from rural private wells in the Annapolis Valley study area are displayed in Figure 1.

The Kolmogorov-Smirnow goodness-of-fit test and visual inspections of histograms were used to assess normality. The distribution of the data from municipal water supplies within the Annapolis Valley study area, data from HRM, and data from rural wells, were all significantly different from normal ($p < 0.01$ for all data sets). Square-root, cube-root and natural logarithm (n+1) of transformations were used to normalize the data in order to meet the assumptions of linear modelling.

The distribution of the data from municipal water supplies within the Annapolis Valley study area were significantly different from normal following square-root ($p = 0.01$), ln(n+1) ($p = 0.01$) and cube-root transformation ($p = 0.02$). However, the histogram of the data appeared to be the most normal with the cube-root transformation. The distribution of the data from HRM were significantly different from normal following ln

(n+1) transformation, but the data were normally distributed following square-root ($p = 0.15$) and cube-root ($p = 0.15$) transformations. The cube-root transformation improved the distribution of the data the most (making it more normal). The distribution of the data from rural wells was significantly different from normal after all transformation ($p = 0.01$ for all transformations), but again, the distribution of the data was the most normal following the cube-root transformation. Therefore, the cube-root transformed data were utilized in subsequent linear modelling for all three groups, and back-transformed for interpretation.

4.2.2 Linear Mixed Effects Models of Nitrate Concentrations from Municipal Water Supplies

The linear mixed effects model for municipal water supplies in the Annapolis Valley described a large amount of the variation in nitrate concentrations ($R^2 = 0.74$, $p = 0.002$). There were significant differences in nitrate concentrations between municipalities ($p < 0.0001$), but not between months ($p = 0.92$) or years ($p = 0.38$). Therefore, determining an exposure map for different months or years was not deemed necessary, and so a single map representing nitrate in municipalities within the study area was created taking the median of the concentrations for samples taken from these municipalities. A map of the verified boundaries of the municipal water supply distribution systems is shown in Map 1.

Within HRM, the linear mixed effects model showed that there were no significant differences in nitrate concentrations by water treatment plant ($p = 0.15$), month ($p = 0.79$)

or year ($p = 0.19$). Therefore, a single nitrate estimate was used within HRM over the study period.

4.2.3 Linear Mixed Effects Models of Nitrate Concentrations from Rural Wells

The linear mixed effects model including nitrate concentrations from rural wells showed that there were significant differences in nitrate concentrations between wells ($p < 0.0001$) and between months ($p < 0.0001$), but not between years ($p = 0.45$). However, when each variable was considered independently in a linear model, measurements from wells described most of the variation in nitrate in rural areas ($R^2 = 0.75$, $p < 0.0001$), while month ($R^2 = 0.01$, $p = 0.12$) and year ($R^2 = 0.0002$, $p = 0.61$) described little of the variation in nitrate concentrations. Compared to the linear model evaluating the independent effects of well on nitrate concentration, the linear mixed effects model did not describe any additional variation in nitrate in rural areas ($R^2 = 0.76$). Therefore, determining an exposure map for different months or years was not deemed necessary, and so a single map representing rural nitrate estimates was created through the interpolation process. A map of the locations of the 140 private wells used to interpolate the ordinary kriging model map is shown in Map 2.

4.2.4 Exposure Assessment

Given the paucity of water quality available for this project, as well the epidemiological, rather than hydrogeological focus of the project, geostatistical methods were chosen in this study to generate an interpolation of groundwater nitrate concentrations in rural areas. The prediction errors associated with various groundwater nitrate interpolations are

displayed in Table 2. The options selected in ArcGIS™ to generate each of the interpolations are also listed in Table 2. The first option listed for each interpolation examined is the interpolation technique: either IDW or ordinary kriging. For models generated using IDW, the size of the neighbourhood refers to the number of nearby wells (typically a range) that were used to estimate the nitrate concentrations at all points for which they were unknown. In some cases, an option was selected to have the neighbours coming evenly from four quadrants (divided at 45°, 135°, 225° and 315°) surrounding each point at which the nitrate concentration was unknown. When this option was selected, the word "divided" is listed following the number of neighbours used to generate the model. For models generated using ordinary kriging, the second option listed is the theoretical variogram selected, either spherical or exponential, which assume slightly different patterns in the decrease of autocorrelation with increased distances between points. The third option listed for models generated using ordinary kriging is the size of the neighbourhood, as was described for IDW.

The nitrate interpolation for the remainder of the analyses was created using ordinary kriging, with an exponential variogram and with a neighbourhood size of 3-5. Neighbours were evenly selected from four quadrants (divided at 45°, 135°, 225° and 315°) surrounding each unknown. This interpolation was selected because it had the lowest mean error, the mean standardized error close to zero, the root mean square standardized error close to one, and the root mean square error is close to the average standard error. The map generated by this model is shown in Map 3. The validation map generated using only 90% of the nitrate sampling locations is shown in Map 4. There

were only a few visible differences between the map generated with all data points and that generated with only 90% of the data points, suggesting that the chosen model was not affected greatly by individual points, and is a valid approximation of groundwater nitrate concentrations for the area. The general patterns of nitrate concentrations from the selected interpolation were also similar to the DRASTIC model by Atari (11) and to the nitrate concentrations presented in the Hydrogeological Atlas of the Annapolis Valley (97).

The map used for subsequent analyses is shown in Map 5. This map comprises of nitrate concentration estimates derived from the selected interpolation model in rural areas median nitrate concentrations from public water supplies in municipal areas.

4.3 Descriptive Statistics for Health Data

4.3.1 Frequencies of Congenital Anomaly Diagnoses

Congenital anomalies of the central nervous system were the most common, affecting 47% of cases in the study population: 40% of cases prior to 1998 and 61% of cases after 1998 (Table 3). Among those study participants diagnosed with a major congenital anomaly, the frequencies of congenial anomalies in by major body system were rank-ordered as follows: (1) central nervous system, (2) musculoskeletal system, (3) gastrointestinal system, (4) cardiovascular system, (5) the inguinal canal, and (6) chromosomal anomalies (Table 3).

4.3.2 Distributions and Univariate Associations for All Variables Included in the Study

For cases and controls from the Annapolis Valley, the distribution of study participants for all covariates, as well as the univariate associations between incidence of congenital anomalies and all covariates, are presented in Table 4. Because cases and controls were matched based on sex, season of conception and year of conception, the distribution of cases and controls was nearly identical for these variables ($p > 0.8$ for all variables). Case mothers were significantly more likely than control mothers to be < 20 years old ($p < 0.0001$). Case mothers were also significantly more likely to be smokers ($p < 0.001$). Case mothers were significantly less likely than control mothers to have had one or more previous pregnancies ($p = 0.01$). There was no significant difference in drinking-water nitrate exposure level between cases from the Annapolis Valley and controls from the Annapolis Valley ($p = 0.89$).

For cases from the Annapolis Valley and controls from HRM, the distribution of study participants for all covariates, as well as the univariate associations between incidence of congenital anomalies and all covariates, are presented in Table 5. Case mothers were significantly more likely to be < 20 years than control mothers from HRM ($p < 0.0001$). Case mothers were also significantly more likely to be smokers ($p < 0.0001$). Case mothers were significantly less likely than control mothers from HRM to have taken a folic acid supplement ($p = 0.01$). Logistic regression models to determine the association between congenital anomalies and drinking-water nitrate exposure level could not be created using controls from HRM because there was no variation in drinking-water nitrate exposure level among controls from HRM.

4.3.3 Distribution of Covariates Among Cases Stratified by Maternal Age at Conception

Table 6 shows the distribution of maternal demographic characteristics, risk factors, water quality variables and nitrate exposure by maternal age at conception among the mothers of cases of congenital anomalies in Annapolis Valley. Mothers < 20 years old were significantly more likely to be smokers than older mothers ($p = 0.03$) and significantly more likely to have not experienced any previous pregnancies ($p = 0.01$). There were no differences in drinking-water nitrate exposure based on maternal age.

4.4 Statistical Modelling

4.4.1 Models Including All Study Participants

Table 7 shows the construction of logistic regression models for the comparison of cases to controls from the Annapolis Valley. Table 8 shows the construction of logistic regression models comparing cases to controls from the Annapolis Valley, without variables representing folic acid supplementation and pre-pregnancy weight. These variables were removed due to large numbers of missing entries for these variables. The omission of these variables did not affect overall trends in the data (shown below). Therefore, models that omitted folic acid supplementation and pre-pregnancy weight were used as the basis for subsequent analyses.

All models using controls from the Annapolis Valley showed an increased risk of congenital anomalies associated with smoking and with maternal age ≥ 35 years at the time of conception. A significant protective effect associated with one or two previous

pregnancies was also observed. Drinking-water nitrate exposure levels above 1 mg/L were slightly positively, though non-significantly, associated with incidence of congenital anomalies. Including demographic characteristics and maternal risk factors greatly improved overall significance of the model (p=0.19), though the significance of the model decreased slightly upon the inclusion of water quality variables and nitrate (p=0.22).

Table 9 shows the construction of logistic regression models comparing cases from the Annapolis Valley study area to controls from HRM, including all variables. Table 10 shows the same logistic regression models, omitting variables representing folic acid supplementation and pre-pregnancy weight. Omitting these variables changed the overall model parameter estimates very little. Therefore, these variables were excluded from subsequent analyses. The only difference between cases from the Annapolis Valley study area and controls from HRM was a significant risk of congenital anomalies associated with smoking. Adjusting for maternal risk factors improved the overall significance of the model (p= 0.54).

The model parameter estimates generated using controls from the Annapolis Valley study area, and controls from HRM were generally consistent. However, there were significant associations between congenital anomalies and maternal age $\geq$ 35 as well as parity for controls from the Annapolis Valley, but not for controls from HRM. Both the magnitude and the direction of the associations with maternal age $\geq$ 35 and parity were different for the two control groups.

4.4.2 Subset Models for Cases and Controls Using Groundwater

Table 11 compares the logistic modelling results generated using all study participants from the Annapolis Valley, to the results generated using only a subset of participants served by groundwater sources. As all study participants in the study area served by surface water were from Kentville, and they all had a municipal source of water with the same nitrate exposure designation, a subset analysis was not conducted for those served by surface water. The general influences of maternal age, parity and smoking were similar for groundwater users compared to all study participants. The increased risk of congenital anomalies associated with nitrate exposure was slightly higher for groundwater users, though it remained statistically non-significant. However, the overall significance of the model including only those served by groundwater was improved compared to the entire study population ($p=0.07$).

4.4.3 Subset Models for Cases and Controls Using Municipal Water or Rural Wells

Table 12 compares the results of logistic regression modelling using all study participants from the Annapolis Valley to the subset served by municipal public water supplies and the subset served by rural private wells. Among study participants served by municipal water supplies there was a significant protective effect associated with one or two previous pregnancies. There was still a slightly increased risk of congenital anomalies associated with nitrate levels above 1mg/L, though the odds ratio was lower in magnitude than that determined using all study participants. When only those participants served by private wells were included in the analysis, there were significant risks associated with

maternal age ≥ 35 years and smoking. The magnitude of the positive association between nitrate and congenital anomalies was increased, though the relationship was still not statistically significant. The model using all study participants had a lower p-value than either model using only a subset of study participants (p=0.22), though the significance of these different p-values could not be directly compared due to different sample sizes.

4.4.4 Subset Models For Cases and Controls Conceived Before or After the Fortification of Grain with Folic Acid in 1998

Table 13 shows the results of logistic regression modelling including all variables after the stratification of the study participants from the Annapolis Valley study area by conception before, or after, folic acid fortification in Canada in 1998. Among those conceived after folic acid fortification, significantly increased risks of congenital anomalies associated with maternal age > 35 years and smoking were observed. There was also a significantly decreased risk of congenital anomalies associated with mothers having one or two previous pregnancies. After the onset of nation-wide folic acid fortification of grain products, there was also a significantly increased risk of congenital anomalies associated with exposure to 1-5.56 mg/L nitrate in drinking-water. The logistic regression model generated using only study participants conceived after folic acid fortification has the lowest log-likelihood p-value of all regression models generated (p = 0.04).

CHAPTER 5: DISCUSSION

5.1 Major Findings

This study builds on the existing literature on the association between congenital anomalies and drinking-water nitrate in several ways. It is one of very few studies to have used population-based data that includes information on fetuses with a prenatal diagnosis of congenital anomaly who underwent spontaneous pregnancy loss or second trimester termination. This study also controlled for more maternal demographic and risk factors than previous studies. Furthermore, it is the first study, to our knowledge, to use GIS methods to assign individual nitrate exposure levels to study participants living in rural areas.

5.1.1 Water Quality and Nitrate Data Analysis

Examination of the nitrate concentrations taken from municipal water supplies and rural wells in this study showed that although there were significant differences between months and years, most of the variation between concentrations is attributable to measurement site. This is in agreement with a previous analysis of the temporal trends in nitrate concentration within the wells from the Annapolis Valley study area (26). These results also agree with a study in Prince Edward Island that found that 92% of the variation in groundwater nitrate concentrations was attributable to differences between wells over a 3-year period, and that 55% of the variation was attributable to differences between wells over a 16-year period (35).

Too little data were available to reliably assess the temporal stability of drinking-water nitrate concentrations over the entire study period from 1987 to 2006. Previous research using nitrate concentrations from the same wells in 1989, 1999 and 2000 found that nitrate concentrations in 1999 were significantly higher than those in 1989 or 2000, though there were no significant differences between 1989 and 2000 (1). Other examinations of long-term trends in groundwater nitrate show mixed results (29-31,33).

5.1.2 Exposure Assessment

The interpolation method with the lowest prediction errors was generated using ordinary kriging. This agrees with two comparisons of geostatistical techniques conducted using simulated data sets: one showed that kriging methods were better interpolators than inverse distance weighting, and that ordinary kriging outperformed universal kriging, regardless of surface type, sampling pattern, noise and spatial correlation. The other comparison showed that ordinary kriging out-performs IDW and Thiessen polygons, but not triangulated irregular networks (109,110).

The interpolated nitrate concentrations at all points for which the actual nitrate concentration was unknown were based on the nitrate concentrations measured at between 3 and 5 nearby wells. These nearby wells were selected evenly from four quadrants surrounding each point, with the quadrants divided approximately in northeasterly, southeasterly, northwesterly and southwesterly directions. The face validity of this model is supported by the hydrogeology of the study area because the general flow of groundwater in the study area is in a northeasterly direction. Water flows from the

edges of the Valley into the centre, and then along the centre of the Valley into the New Minas Basin (97). Therefore, the selection of the nearby wells from these quandrants ensured that the interpolation at each point was based on influences from both sides of the Annapolis Valley (Map 6).

5.1.3 Descriptive Statistics for Health Data

Congenital anomalies of the CNS were the most commonly diagnosed among study participants. The next most common types of diagnoses were the musculoskeletal system and gastrointestinal system. Previous studies examining the relationship between congenital anomalies and drinking-water nitrate found associations between CNS anomalies and nitrate (5,6). However, another Nova Scotia study of congenital anomalies by Dodds *et al.* (2) observed that they occurred by body system in the following order: (1) cardiovascular system, (2) musculoskeletal system, (3) chromosomal anomalies, (4) ears, eyes, nose and throat, (5) genitourinary system, and (6) central nervous system (2). Differences in the rank-ordering of congenital anomaly diagnoses by body system may be due to genuine differences in the incidence of different types of congenital anomalies within this study population compared to other populations in Nova Scotia.

Both this study and the study by Dodds *et al.* (2) used the NSAPD to ascertain information. However, this study also included cases in the FADB, which allowed the inclusion of fetuses with a prenatal congenital anomaly diagnosis who did not survive to 20 weeks or were electively terminated. There may be differences in the types of congenital anomaly diagnoses among those cases from the FADB compared to those

from the NSAPD that were reflected in the differences in the types of congenital anomalies observed between this study and the other Nova Scotia study.

In this study, CNS anomalies were the most common type of anomaly diagnosed both before and after folic acid fortification. Anomalies of the CNS represented greater proportion of diagnoses after folic acid fortification compared to before folic acid fortification. Previous research has shown that folic acid fortification substantially reduced the incidence of NTD in Nova Scotia (111). However, NTD are only one group of CNS anomalies, therefore a reduction in incidence of NTD may not have been observable because CNS anomalies as a whole were tabulated in this study.

Furthermore, in this study the ascertainment of cases improved with the inclusion of cases with prenatally diagnosed anomalies that did not survive until 20 weeks or were electively terminated when the FADB was established in 1992. Research in Hawaii found that the prevalence rates of selected congenital anomalies increased upon improvements in prenatal diagnosis, and the inclusion in databases of second trimester terminations of pregnancy after diagnoses of congenital anomalies (112). Compared to other types of congenital anomalies, the prevalence rates of NTD increased the most when second trimester terminations of pregnancy for congenital anomalies were included in prevalence rate estimations (112).

Case ascertainment in the FADB likely improved between 1992 and 1998. Improved prenatal screening and diagnosis of congenital anomalies in Canada led to a 578%

increase in second-trimester pregnancy terminations in Canada between 1991 and 1998 (113). In 1998, the inception of the Nova Scotia Civic Address System increased the proportion of cases in the FADB that could be included in this study as infants and fetuses could only be included as study participants if their maternal addresses at the time of delivery could be linked to a latitude and longitude. The standardized address system enabled a much greater proportion of the addresses retrieved from the patient charts of cases in the FADB to be linked to a latitude and longitude.

Therefore, over the entire time period of the study, three changes occurred that likely increased the ascertainment of cases with prenatal diagnosed congenital anomalies in this study. These changes may have had differential effects by type of congenital anomaly, likely increasing the ascertainment of cases with CNS anomalies over time. Over the same time period, the onset of folic acid fortification would have decreased the actual incidence of NTD in the study population. Increases in the case ascertainment of CNS anomalies in general may have been sizeable enough to mask the decrease in incidence of NTD over the study period.

5.1.4 Statistical Modelling

Logistic regression models examining the effects of all of the covariates, including water quality variables and nitrate, were not strongly influenced by the omission of variables representing folic acid supplementation and pre-pregnancy weight, despite evidence that suggests these factors are associated with incidence of congenital anomalies. The direction of associations between congenital anomalies and other covariates did not

change upon the omission of folic acid supplementation and pre-pregnancy weight, though more covariates were statistically significant due to narrower 95% confidence intervals. Therefore, the models without folic acid supplementation and pre-pregnancy weight are likely better indicators of the associations between incidence of congenital anomalies and covariates. These models are discussed below, with the exception of those results specifically related to folic acid supplementation and pre-pregnancy weight.

5.1.5 Statistical Modelling of Demographic Characteristics

When controls from the Annapolis Valley were used in the analyses, crude odds ratios showed no differences in incidence of congenital anomalies by maternal age. After adjustment for maternal demographic traits and risk factors there was a significant increased risk of congenital anomalies associated with maternal age ≥ 35 years. Using controls from HRM, no association between congenital anomalies and maternal age was observed, even after controlling for other covariates. Some previous studies have shown that congenital anomalies are associated with both increased maternal age and young maternal age, while others have shown associations only between congenital anomalies and increased maternal age (57,74,75,114). It has been suggested that differences in incidence of congenital anomalies according to maternal age could be due to variation in medical care between age groups, physiological changes that occur with increased age, or cumulative lifetime exposure to teratogens (74,75).

It has also been suggested that some differences could be related to the distribution of risk factors for congenital anomalies between age groups (74). This study found that

among case mothers there were significant differences in smoking and parity by maternal age. This is consistent with another Nova Scotia study that found that women under 20 years of age are more likely to smoke during pregnancy (58). Discrepancies in the results generated using the controls groups from the Annapolis Valley and HRM could be a reflection of differences in the distribution of maternal risk factors between the two areas.

This study found that there is a significant protective effect against congenital anomalies associated with one or two previous pregnancies when cases were compared to controls from the Annapolis Valley, but not when compared to controls from HRM. Though nearly all epidemiological studies of congenital anomalies show associations with parity, and control for parity, none were found that gave a clear rationale for the potential confounding effect of parity in such studies. Previous studies have found that primiparity, especially in combination with older age and higher pre-pregnancy BMI, is a significant risk factor for congenital anomalies (70,115). The relationship between congenital anomalies and parity may further be confounded by the use of assisted reproductive technology, which may be associated with increased rates of congenital anomalies (116,117). Differences in the associations between congenital anomalies and parity found in this study when different comparison groups were used suggest that some type of neighbourhood-level effects could influence the association. Neighbourhood-level effects could also feasibly influence the age of primiparious women, BMI and the use of assisted reproductive technologies, though insufficient evidence exists to provide an explanation for the observed association between congenital anomalies and parity using controls from the Annapolis Valley.

5.1.6 Statistical Modelling of Maternal Risk Factors

This study found that smoking is a significant risk factor for congenital anomalies, regardless of which control group was used for analysis. Some previous studies have shown positive associations between smoking and congenital anomalies (59), though others have shown no association (60,118). Typically, studies of congenital anomalies and smoking controlled for demographic characteristics but not other maternal risk factors for congenital anomalies. However, one study that did control for some maternal risk factors found that CNS anomalies were significantly associated with maternal smoking (59). It is possible that this study was less encumbered by imprecision than others studies, as adjustments were made for several maternal risk factors using systematically collected information with increased data precision, contributing to the observed positive association between smoking and congenital anomalies.

In Nova Scotia, women are more likely to smoke during pregnancy if they are less than 20 years of age, are not married, did not take prenatal classes or if they have had 3 or more previous pregnancies (58). Smoking during pregnancy has been associated with increased alcohol consumption and lower levels of education in other jurisdictions (60). It is possible that the positive association between congenital anomalies and smoking observed in this study, which contradicts previous studies that have shown no association (60,118) is the product of residual confounding. Despite controlling for a wide array of covariates, this study did not attempt to control for factors such as education, household

income, marital status and alcohol consumption that may also be related to smoking, drinking-water nitrate exposure and incidence of congenital anomalies.

There was no difference in the crude odds of congenital anomalies among participants conceived before and after folate fortification in this study, regardless of comparison group used. The protective effect of folic acid against neural tube defects is well established (61). Previous work in Nova Scotia has also shown that the incidence of NTD was reduced after widespread folic acid fortification (111). It is likely that the onset of folic acid fortification showed no protective effect against congenital anomalies in this study because the analysis examined all major congenital anomalies, rather than exclusively NTD, which are specifically associated with folic acid intake.

No association was observed between folic acid supplementation and incidence of congenital anomalies using the control group from the Annapolis Valley. Analysis using the control group from HRM showed that folate supplementation had a significant protective effect after adjustment for sex, month of conception and year of conception. However, the protective effect was attenuated and became non-significant after controlling for other maternal risk factors. These results are supported by previous research in Nova Scotia that found that recommendations for folic acid supplementation had only a limited effect on incidence of NTD (111).

This study, as well as the study by Persad *et al.*, (111) relied on self-reported measures of folic acid supplementation available in the NSAPD and the FADB. There may be bias

associated with this variable, especially if women had tendencies towards difficulty in recall or to provide an answer that is different from the truth, and if these tendencies varied between women in a systematic fashion, such as by control group. If this is the case, it would suggest that this variable more accurately represents awareness of the folic acid recommendations or general healthy behaviour, rather than actual folic acid supplement intake. This hypothesis is supported by the attenuation of the effects of folic acid supplementation after controlling for other maternal risk factors. Therefore, differences in the association between congenital anomalies and folic acid supplementation based on the control group used could be due to differences in other behaviours or neighbourhood characteristics between the Annapolis Valley and HRM control groups.

This study found no significant associations between pre-pregnancy weight and congenital anomalies, which is different than previous studies that have shown significant positive associations between congenital anomalies and maternal overweight and obesity (62-66), though not between congenital anomalies and maternal underweight (64,66). This study only examined self-reported pre-pregnancy weight, with no adjustment for height. A large proportion of reports of pre-pregnancy weight were also missing. Other studies used body mass index derived from self-reported measures of body weight and height for comparison (62,63,65,66). The cut points selected in the current study were loosely tied to traditional BMI cut-offs for underweight and overweight based on the average height of Canadian women. Therefore it is likely that women taller than average were erroneously classified as overweight. Considerable misclassification of pre-

pregnancy weight may have attenuated an actual association between overweight and congenital anomalies in this study.

After controlling for maternal demographic characteristics and risk factors, there was a non-significant increased risk of congenital anomalies associated with pre-existing maternal diabetes and gestational diabetes. Congenital anomalies are positively associated with poor glucose control (67). Hyperglycemia, hypoxia, ketone and amino acid abnormalities, the over-production of oxygen free-radicals, and glycosylation of proteins are postulated causal pathways (67,68). Previous studies have shown strong positive associations between congenital anomalies and pre-conceptional diabetes (65,68,69). Gestational diabetes has also been linked to the development of congenital anomalies, though it is plausible that these associations are in part a reflection of pre-existing type 2 diabetes diagnosed only in pregnancy (68). It is possible that a significant positive association was not observed in this study because of the small number of women with diabetes included in the study.

This study did not find an association between thyroid disease and congenital anomalies. The odds ratios were not consistent in direction, and had very large confidence intervals. Furthermore, too few diagnoses of thyroid conditions were made to enable the calculation of odds ratios in analyses with smaller sample sizes. Similarly, literature relating congenital anomalies to thyroid disease is mixed. Though Health Canada (1) listed thyroid disease as a risk factor for congenital anomalies, very few studies have examined the association, and no studies could be found that showed a positive association (72,73).

5.1.7 Statistical Modelling of Water Quality Variables and Nitrate

This study showed a positive, but not statistically significant, association between congenital anomalies and drinking-water nitrate when all study participants were included in the data analysis. Most previous studies examining the relationship between congenital anomalies and drinking-water nitrate and congenital anomalies have found positive and sometimes statistically significant association between drinking-water nitrate and congenital anomalies (3,5-7,54) . The majority of previous studies reported odds ratios similar in magnitude to the ones presented in this study, though they also examined much higher drinking-water nitrate concentrations. In order to create nitrate exposure levels across which the study controls were relatively evenly distributed, the cut-points were assigned at 1 mg/L and 5.56 mg/L. The most comparable study, by Cedergren *et al.* (7), examined nitrate concentrations greater than 2 mg/L, and reported an odds ratio of 1.18 (95% CI 0.97-1.44), slightly lower than the odds ratios reported in this study.

This study did not find evidence for a dose-response relationship between incidence of congenital anomalies and drinking-water nitrate exposure level; the odds ratio was slightly greater for the lower exposure category (1-5.56 mg/L) than for the higher exposure category (>5.56 mg/L). This may simply be a reflection of a very small number of study participants exposed to drinking-water nitrate concentrations >5.56 mg/L. It may also be the result greater variability in the drinking-water nitrate concentrations in areas prone to higher nitrate concentrations, resulting in poorer quality drinking-water nitrate concentration estimates in these areas. Lastly, the lower association with congenital

anomalies observed at higher nitrate concentrations may be a spurious result due to increased bottled water consumption by individuals living in areas with drinking-water with high nitrate concentrations.

When analyses were repeated omitting study participants from Kentville, the only source of surface water in the Annapolis Valley study area, the magnitude of the association between nitrate and congenital anomalies was increased though it remained non-significant. Due to the small number of individuals served by surface water in the Annapolis Valley study area, no meaningful inference can be made in the study into the effects of groundwater, or groundwater and nitrate, on incidence of congenital anomalies. Three previous studies suggested that a significant positive association between congenital anomalies and drinking-water nitrate exists only upon the consumption of groundwater, or that the association is greater upon the consumption of groundwater (3,5,6).

Upon stratification by rural and urban water sources, the magnitude of the association between congenital anomalies and drinking-water nitrate after adjustment was increased for rural residents. The magnitude of the association was slightly decreased for municipal residents. Only two previous studies on congenital anomalies and drinking-water nitrate included both urban and rural participants (3,5). The study by Dorsch *et al.* (3) found a significantly increased risk of congenital anomalies associated with certain rural water sources, while the study by Arbuckle *et al.* (5) found a non-significant risk associated with public water supplies.

Since the observed association between congenital anomalies and nitrate differs based on water source, it is possible that the association may be modified or confounded by another component of drinking-water. A positive association between congenital anomalies and drinking-water disinfection by-products in municipal water supplies is well-established (119). It is possible that some congenital anomalies in municipal areas are attributable to disinfection by products, thereby contributing to a lower association between congenital anomalies and nitrate in these areas. Conversely, it is possible that the elevated risk associated with nitrate in rural areas relative to municipal areas is due to an association between congenital anomalies and an unknown contaminant in rural water supplies that is correlated with nitrate contamination. For example, in the same Annapolis Valley study area used for this project, 71% of wells with elevated nitrate levels also contained pesticides, most frequently Atrazine (10). Atrazine has been positively associated with the development of abdominal wall defects (53).

5.2 Study Strengths

A workgroup report prepared following a symposium on drinking-water nitrate and health at the 2004 meeting of the International Society for Environmental Epidemiology emphasized the need to study users of private wells (8). Furthermore, this report asserted that GIS is a promising approach for better estimations of nitrate exposure risk (120). A review paper published in 2006 echoed the need for further investigation into the relationship between drinking-water nitrate and health among those served by private

water supplies (9). This report also recommended attempts to control for more potential confounding factors (9).

The primary strengths of this study are that a number of participants were included that live in rural areas and were served by private wells, and that individual-level estimates of drinking-water nitrate exposure were derived using GIS analysis. The vast majority of epidemiological studies that have been undertaken using GIS looked only at aggregate data (121). Using individual-level data reduces the potential for exposure misclassification and makes it more likely that the true association between the exposure and the outcome will be observed through the study. Individual level exposure estimates, as were used in this project, can reduce recall bias in research (87).

Another major strength of the study is that participants were selected from population-based databases that included data on fetuses with a prenatal diagnosis of congenital anomaly who underwent spontaneous pregnancy loss before 20 weeks of gestation or second trimester termination. Common sources of error in the collection and coding of data for population-based perinatal databases are incomplete participation by hospitals, differing means of diagnosis, discrepant criteria for the diagnosis of congenital anomalies and few records on therapeutic abortions (122). These errors are overcome in the NSAPD and the FADB as all hospitals in the province participate in the databases, many non-hospital births are included, diagnosis criteria are standardized and information is available on second trimester terminations (1). The means by which information

collected for the NSAPD and the FADB are well described, and re-abstraction studies indicate that the data contained in the NSAPD are reliable and of good quality (106,107).

The information on maternal demographic and risk factors contained in the NSAPD and the FADB enabled controlling for a large number of covariates in this study, and met the third workgroup recommendation listed above. This enabled a more precise evaluation of the association between nitrates and congenital anomalies.

5.3 Study Limitations

A potential weakness of this study is that the 140 Kings County wells from which the GIS estimates of nitrate concentration were derived were chosen among the 237 wells selected for research by Briggins and Moerman (10) using the DRASTIC risk assessment model may have introduced a bias (10,26). More wells with a higher DRASTIC rating were selected relative to wells with a lower DRASTIC rating (10). The means by which wells were selected for sampling may have led to an overestimation of nitrate concentrations in wells within the Annapolis Valley study area. If there was a widespread overestimation of nitrate concentrations in rural wells, differences in the association between congenital anomalies and nitrate for rural and urban areas could have been exaggerated. However, there appears to be no relationship between rural and urban residence and incidence of congenital anomalies, therefore an overestimation of nitrate concentrations in rural wells likely did not bias the study results.

Another limitation of the study is that ordinary kriging generates a rectangular surface bounded by the four sampling points extending the farthest in all four directions. To maximize power in the study, it was necessary to select study participants from all regions within the study area. In order to do so, the interpolation was extended to cover the entire study area. In general, in areas with few sampling points, geostatistical methods, such as the one used, provide poor estimates, especially relative to process-based methods (92). However, approximately 72% of all civic address points identified in the Annapolis Valley study area fall within the initial rectangular interpolation area or in the municipality of Greenwood, which is the only municipality outside the interpolation area. Approximately 61% of civic addresses in the study area served by private wells are located in the initial rectangular interpolation area. This suggests that the interpolation method used is appropriate for most of the study participants.

The most common means of geocoding, estimating the position of houses based on house number and street length, is subject to a substantial amount of error and can induce bias in epidemiological studies (123). Access to the Nova Scotia Civic Address File eliminated some error in geocoding, as each structure in Nova Scotia was measured with a handheld GPS unit, resulting in estimates of the centre-points of each structure that are accurate within 2.5m (124). However, for homes served by private wells the aquifers providing drinking-water were not directly under the centre of the building. Therefore, there is likely some misclassification of drinking-water nitrate estimates resulting from distance between the homes and the water sources.

Using only estimates of drinking-water nitrate at women's homes at the time of delivery to estimate their overall drinking-water nitrate exposure likely led to some misclassification of drinking-water nitrate exposure. Statistics Canada data indicate that 19% of Kings County residents aged 15-29 moved in the year preceding the 2001 census, while 10% of residents aged 30-44 moved in this year (125). Approximately 7% of all Canadians moved in the year preceding the 2006 Census. American studies have found that 20-33% pregnant women move between conception and delivery, though a Canadian study found that only 13% of pregnant women moved (126-129). No differences in mobility were noted between women who gave birth to a child with, or without, congenital anomalies (126,127). Therefore, this project, like other epidemiological studies involving pregnancy outcomes and exposures tied to geography, was likely subject to considerable non-differential misclassification possibly creating a bias towards the null (126).

Another limitation of the study is a lack of information pertaining to where women consumed tap water, and how much tap water they consumed relative to bottled water. A large proportion of pregnant women in the study area could be consuming tap water away from home. The 2001 census also showed that 55% of women living in Kings county work outside the home, though most work and live in the same census subdivision, or commute from rural areas into Kentville or Wolfville for work (125). A study in the southern United States showed that each day pregnant women consume on average 1.3L of tap water at home, compared to 0.4L of tap water at work (130). The same study showed that pregnant women consume and average of 0.6L of bottled water each day.

Geographic location was significantly associated with amount of tap water and bottled water consumed (130). This suggests that while using only home tap water to estimate drinking-water nitrate exposure is far from representative of actual drinking-water nitrate exposure, home tap water is probably the best single means of estimating drinking-water nitrate exposure. For most study participants, home tap water will confer the same, or a slightly higher, likelihood of nitrate exposure as work tap water. All tap water will contain more nitrate than bottled water. Therefore, it is possible that the nitrate exposure attributable to the consumption of drinking-water by study participants was overestimated in this study.

Drinking-water is not the only source of nitrate or nitrite ingestion. Both compounds can also be ingested via food and drugs (15,36,56,77). As individuals were not contacted, it is not known how much nitrate they were exposed to from these sources. However, previous research found that drinking-water nitrate is often the most important contributor to adults' nitrate ingestion (56). A study in New Zealand showed that drinking-water alone can contribute to nitrate exposure of up to 72% of the national Acceptable Daily Intake (ADI) for individuals with high levels of nitrate in their water sources, but that drinking-water nitrate contributes only a fraction of daily nitrate exposure for most individuals (77). As the concentration of nitrates in drinking-water increases, the relative contribution of nitrate from drinking-water also increases. Drinking-water below the nitrate MAC in Canada usually accounts for 30% of an adult's total nitrate load, though if the nitrate level exceeds the MAC, drinking-water can account for 70-80% of an adult's total nitrate load (56). For study participants who live in

areas with relatively high drinking-water nitrate levels the drinking-water nitrate concentration estimates used in this study are likely good indicators of total nitrate exposure. For others, drinking-water nitrate estimates are likely a poor indicator of total nitrate exposure.

Previous literature has suggested that there is an association between congenital anomalies, especially NTD, and socioeconomic status (131). It has been suggested that associations between negative health outcomes and socioeconomic status are manifestations of associations between negative health outcomes and other factors, such as smoking (131). For example, education can influence health decision-making and occupation affects workplace exposures and social networks (131). However, low parental socioeconomic status and low neighbourhood socioeconomic status are associated with increased incidence of NTD after controlling for other maternal risk factors for congenital anomalies (132). Individual and household socioeconomic status has also been positively associated with NTD and other congenital anomalies after controlling for other risk factors for congenital anomalies (131). Furthermore, socioeconomic characteristics such as income and education are likely associated with neighbourhood of residence, private drinking-water monitoring and bottled water consumption. Since there may be an association between congenital anomalies and socioeconomic status (both at individual and neighbourhood levels), and an association between congenital anomalies and drinking-water nitrate consumption this study may have been limited by residual confounding.

Previous research in the Annapolis Valley study area has found that those wells that have drinking-water nitrate levels that exceed the MAC are more likely to contain fecal coliform bacteria and pesticides (10). In general, a high nitrate concentration in a well is indicative of the additional presence of other contaminants. This study may be limited by residual confounding because the presence of other drinking-water contaminants that may be correlated with high nitrate concentrations were not evaluated. For example, pesticides exposure may be associated with increased incidence of congenital anomalies and is also positively correlated with nitrate concentrations (10, 53).

Another limitation of this study was that analyses were not sub-divided according to specific type of congenital anomalies, as has been recommended in previous research (8,9). Environmental teratogens are expected to exert specific effects, contributing to the development of a relatively narrow range of congenital anomalies (50). Therefore, investigations into associations between potential teratogens and all anomalies, such as the current study, may be biased towards the null due to differing associations between nitrate and certain types of congenital anomalies.

5.4 Policy Implications

This study found that over the period from 1998 to 2000 there was a significant increase in the incidence of congenital anomalies among participants exposed *in utero* to drinking-water nitrate levels at just 10% of the Canadian MAC. The results of this study support the existence of the current drinking-water nitrate MAC in Canada, and suggest that it may be necessary to consider lowering the drinking-water nitrate MAC.

The drinking-water nitrate MAC was established 50 years ago in response to observed risks associated with infant methemoglobinemia (49,133). The appropriateness of the current Canadian MAC of 10 mg/L has been questioned by several researchers who suggested that there is insufficient evidence relating nitrate to negative health outcomes to justify the current MAC, and that maintaining the current MAC is too costly for small rural communities (133,134). Other researchers have suggested that the existing research on nitrate and health is not sufficiently rigorous to assess associations between nitrate and health, and sufficient evidence exists to suggest that drinking-water nitrate could be hazardous, warranting further research into the potential hazards of drinking-water nitrate and the maintenance of the current MAC (135). These recommendations are more closely aligned with the evidence presented in this study, which support the existence of a MAC of 10mg/L or lower, and reinforce the need for further research.

Given that environmental teratogens act very early in pregnancy to influence the development of congenital anomalies, and half of the pregnancies in Nova Scotia are unplanned, it is important for women to be educated in risk factors for congenital anomalies and other adverse pregnancy outcomes before they consider becoming pregnant. This study found that a large proportion of pregnant women smoked despite existing public health programs recommending otherwise. Furthermore, this study found that women who are pregnant for the first time give birth to children with the highest incidence of congenital anomalies. Therefore, health education prior to conception should be emphasized.

Prior to 2006, Health Canada funded a program called Mothernet, which maintained a prospective database of prescription drug intake and environmental exposures during the perinatal period, and adverse health outcomes, including congenital anomalies (136). However, this program has since been cancelled (137). The re-establishment of a similar program, or the incorporation of measures of more environmental exposures in perinatal databases such as the NSAPD and FADB, would facilitate investigations into potential environmental causes of congenital anomalies.

5.5 Future Research

A natural extension of the current research project would be to repeat the analysis using only those cases which were diagnosed with a congenital anomaly of the central nervous system. Previous literature has suggested that drinking-water nitrate could be specifically related to anomalies of the central nervous system (5,6). However, it is possible that upon stratification by type of congenital anomaly, there will not be sufficient power to detect a difference in incidence of congenital anomalies based on drinking-water nitrate exposure.

It may be worthwhile to undertake a prospective study of drinking-water nitrate on the incidence of congenital anomalies. This would require a large number of participants given the rarity of the outcome. However, since the exposure window and lag period are relatively short, such a study would be feasible. Drinking-water samples could be tested for contaminants other than nitrate, such as pesticides and chlorination by products,

enabling a better understanding of the effects of several potential teratogens through a single study.

In general, future research projects that use GIS to generate individual drinking-water nitrate exposure estimates would benefit from the use of process-based methods of estimation. In addition to providing better reliability in regions with few sampling points, the use of process-based methods would have also enabled the consideration in the nitrate concentration estimates of characteristics that are known to influence groundwater contamination by nitrate. These include: depth to water table and well depth, well construction, land use, agricultural facilities, chemical facilities, surficial geology, bedrock geology, and population density (11,16,23,24,26).

Previous work has estimated that 20-25% of congenital anomalies have multifactorial causes, whereby interactions between genes and the environment contribute greatly to susceptibility to adverse health outcomes (1,138). Improvement in the assessment of the genetic components of diseases can reduce the amount of unattributable variation in environmental epidemiology research, providing the means for a more precise examination of the contribution of environmental exposures to disease susceptibility (138). Combining emerging genetic-epidemiology techniques with improved exposure assessment through GIS has been achieved to examine the relationship between breast cancer and pesticides on PEI (139). Similar techniques could be used to contribute to a better understanding of environmental exposures, such as drinking-water nitrate, that may contribute to the development of congenital anomalies.

Lastly, the exposure assessment model used in this project could be re-used in projects examining the association between drinking-water nitrate and other health outcomes. Drinking-water nitrate has been positively associated with cancers, hypertension, diabetes, methemoglobinemia and a variety of adverse birth outcomes, including intrauterine growth restriction, spontaneous abortion, prematurity (41,41,42,44-48). Within Nova Scotia, a large database of health information, including address information, is available from provincial health insurance records. Examinations between drinking-water nitrate and other health outcomes could be conducted by linking this database with the drinking-water nitrate exposure model created for the current study.

CHAPTER 6: CONCLUSION

Congenital anomalies are common, affecting 2-3% of Canadian births (1). While some anomalies have relatively minor physical effects, others can be severe in nature. In 1995, 1.9 per 10,000 Canadian live births resulted in death due to a congenital anomaly (1). The etiology of many congenital anomalies is poorly understood, though it is believed that up to 12% have direct environmental causes and up to 25% have multifactorial causes (1). Research into potential environmental teratogens is crucial, as exposure to environmental teratogens can be avoided, thereby reducing the burdens associated with congenital anomalies.

This is the first study to emphasize users of private wells, to use GIS to provide individual exposure estimates, and to control for a wide array of maternal risk factors for congenital anomalies. This study also included fetuses diagnosed with congenital anomalies that did not survive to 20 weeks gestation or were electively terminated.

Previous studies have shown positive associations, albeit not often statistically significant association, between drinking-water nitrate exposure and incidence of congenital anomalies (3-7). This study found that after adjustment for maternal demographic traits, risk factors and water-quality variables there is a positive, albeit non-significant, association between exposure to drinking-water nitrate concentrations greater than 1 mg/L and congenital anomalies after adjustment (OR = 1.65, 95% CI 0.83-3.27 for 1-5.56 mg/L; OR= 1.66, 95% CI 0.81-3.42 for > 5.56 mg/L). Among those conceived after folic acid fortification there was a significant positive association between

congenital anomalies and drinking-water nitrate (OR=2.44, 95% CI 1.05-5.66 for 1-5.56 mg/L). However, the time period of the study also coincides with the inception of the FADB enabling the inclusion of cases with prenatally diagnosed congenital anomalies, a period of improved prenatal screening and diagnosis of congenital anomalies, as well as the inception of a standardized address system in Nova Scotia that improved case ascertainment of prenatally diagnosed congenital anomalies in this study. Therefore, it is not known if the observed differences when the entire study period was compared to the last 8 years are due to changes in folic acid fortification or to an increase in the reliability of case ascertainment.

This study provides supporting evidence for of the utility of GIS modelling to develop individual-level environmental exposure estimates, and supports expanded research into the association between congenital anomalies and drinking-water nitrate. It is striking that after controlling for many covariates, and despite the potential for exposure misclassification that likely biased study results towards the null, a positive relationship between drinking-water nitrate and congenital anomalies remained. Future research should stratify analyses based on class of congenital anomalies, as well as reduce misclassification through a prospective study with individual life-histories or tap-water nitrate measurements.

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Table 1. Descriptive statistics of nitrate measurements from municipal water supplies in the Annapolis Valley study area and Halifax Regional Municipality from 1996 to 2003 (all concentrations represent nitrate-nitrogen in mg/L).

Municipalities	n	Minimum	Maximum	Mean (95% C.I.)	Median	Mean of Cube-Root Transformed Measurements (95% C.I.)
All Annapolis Valley Municipalities	53	0.0	10.4	2.03 (1.58 - 2.48)	1.7	1.73 (1.67-1.79)
Canning	24	1.0	2.5	1.63 (1.47 - 1.80)	1.7	1.60 (1.56 - 1.64)
Greenwood	5	0.0	1.0	0.63 (0.12 - 1.14)	0.9	0.46 (0.05 - 0.87)
Kentville	2	0.4	1.0	0.69 (0.00 - 4.28)	0.7	0.66 (0.00 - 2.19)
New Minas	10	1.0	2.9	1.68 (1.92 - 2.07)	1.6	1.64 (1.55 - 1.73)
Port Williams	6	1.4	10.4	5.08 (1.64 - 8.52)	4.6	4.49 (4.08 - 4.90)
Wolfville	6	2.4	3.6	2.77 (2.33 - 3.21)	2.7	2.74 (2.67 - 2.81)
Halifax Regional Municipality	9	0.0	0.1	0.04 (0.00 - 0.01)	0.0	0.01 (0.00 - 0.16)

Table 2. Prediction errors from various interpolation models of rural well nitrate concentrations (n=1113) from July 1999 to February 2000 in the Annapolis Valley and the associated model options. [a]*

Prediction Errors					
	Mean	**Root Mean Square**	**Average Standard**	**Mean Standardized**	**Root Mean Square Standardized**
Model Used for the Analysis					
Ordinary Kriging Exponential variogram 3-5 neighbours divided	0.02	7.59	7.71	0.01	1.08
Other Models Created for Comparison Purposes					
Ordinary Kriging Spherical variogram 2-5 neighbours divided	0.80	8.73	8.81	0.08	1.29
Ordinary Kriging Exponential variogram 2-5 neighbours divided	0.77	8.45	8.86	0.08	1.09
Ordinary Kriging Spherical variogram 5 neighbours divided	-0.03	7.48	7.64	-0.01	1.07
IDW 5-10 neighbours	0.89	8.02			
IDW 3-10 neighbours	0.89	8.02			
IDW 2-5 neighbours	0.97	8.30			
IDW 4-8 neighbours	0.88	8.10			
IDW 4-8 neighbours Search area divided	0.82	7.86			
IDW 10-15 neighbours	0.94	7.98			
Ordinary Kriging Exponential variogram 2-5 neighbours, divided	0.05	7.64	7.84	0.01	1.08
Ordinary Kriging Spherical variogram 2-5 neighbours, divided	0.12	7.95	7.75	0.01	1.28

[a] IDW refers to Inverse Distance Weighting

* Average standard errors, mean standardized errors, and root mean square standardized errors are not generated when IDW is used for interpolation.

Table 3. Frequency of diagnoses of major congenital anomalies in the Annapolis Valley, categorized by body system, over the entire study period (1987-2006), during the period prior to the fortification of food in Canada with folate (1987-1997), and for the period after folate fortification (1998-2006).[±] [*] [a] [b]

	1987-2006 (n=606)			1987-1997 (n=300)			1998-2006 (n=306)		
	n	%	Number per 10,000 births	n	%	Number per 10,000 births	n	%	Number per 10,000 births
All Anomalies	606	100	55	300	100	54	306	100	57
Central Nervous System	286	47	26	98	33	18	188	61	35
Musculoskeletal system	101	17	9	58	19	11	43	14	8
Genitourinary system	50	8	5	25	8	5	25	8	5
Cardiovascular system	47	8	4	26	9	5	21	7	4
Inguinal canal	37	6	3	26	9	5	11	4	2
Multiple anomalies due to chromosomal aberrations and other causes	33	5	3	24	8	4	9	3	2
Eye, ear, nose, throat and mouth	27	4	2	17	6	3	10	3	2
Other (includes gastrointestinal, metabolic, respiratory, and skin)	27	3	2	16	5	3	11	4	2
Missing	53	9	5	53	18	10	0	0	0

* All categories containing fewer than 5 cases were amalgamated.

[a] Both live births and stillbirths, obtained from Vital Statistics, were included in the denominator. Data were only available from 1997 onward. Therefore the number of births from 1988 to 1996 was imputed from the number of births from 1997 to 2006.

Table 4. Distribution of variables between cases of congenital anomalies and controls from the Annapolis Valley, as well as univariate associations between incidence of congenital anomalies and all variables for cases and controls (1987-2006).

	Cases from Annapolis Valley (n=606)		Controls from Annapolis Valley (n=1635)		Cases and Controls from Annapolis Valley (n=2241)	
	n	%	n	%	Crude Odds Ratio (95% CI)	p-value
Sex						0.91
Female	268	48	788	48	1.0	
Male	285	52	847	52	0.99 (0.82- 1.20)	0.91
Missing	53		0			
Season of conception						0.96
Winter	83	15	244	15	1.0	
Spring	139	25	393	24	1.04 (0.76-1.43)	0.63
Summer	162	29	484	30	0.98 (0.73-1.34)	0.87
Fall	169	31	514	31	0.97 (0.71-1.31)	0.70
Missing	53		0			
Year of Conception						0.98
1987-1991	83	15	245	15	1.0	
1992-1996	113	20	342	21	0.98 (0.70-1.35)	0.75
1997-2001	282	51	838	51	0.99 (0.75-1.32)	0.87
2002-2006	75	14	210	13	1.05 (0.73-1.52)	0.67
Missing	53		0			
Maternal age						0.21
< 20	50	9	134	8	1.15 (0.82-1.62)	0.95
20-34	435	79	1339	82	1.0	
≥ 35	68	12	162	10	1.29 (0.95-1.75)	0.28
Missing	53		0			
Parity						**0.01**
0	273	49	687	42	1.0	
1-2	252	46	852	52	**0.74 (0.61-0.91)**	0.29
3+	28	5	96	6	0.73 (0.47-1.14)	0.46
Missing	53		0			
Pre-pregnancy weight (Kg)						
< 50	44	9	129	9		0.55
50- 69	281	59	837	56	1.02 (0.70-1.47)	0.68
≥ 70	155	32	520	35	1.0	
Missing	126		149		0.89 (0.71-1.11)	0.33
Smoker						**<0.001**
No	358	59	1140	70	1.0	
Yes	248	41	495	30	**1.60 (1.32-1.94)**	**<0.001**
Diabetes						0.93
No	586	97	1585	97	1.0	
Gestational	15	2	14	2	1.13 (0.61-2.07)	0.73
Other Diabetes	5	1	3	1	0.97 (0.35-2.69)	0.86
Thyroid disease						0.51
No	604	100	1626	99	1.0	
Yes	2	0	9	1	0.60 (0.13-2.78)	0.51
Folate supplementation						
No	111		317			0.74
Yes	79	58	239	57	1.0	
Missing	416	42	1079	43	0.94 (0.67-1.32)	0.74
						0.07
Folate fortification					1.0	
No	300	50	740	45	0.84 (0.70-1.02)	0.07
Yes	306	50	895	55		
Water source						0.64
Surface	118	19	304	19	1.0	
Ground	488	81	1331	81	0.95 (0.75-1.20)	0.64
Municipal water						0.17
Yes	245	40	609	37	1.0	
No	361	60	1026	63	0.88 (0.72-1.06)	0.17
Nitrate exposure level						0.89
< 1 mg/L	127	21	353	22	1.0	
1-5.56 mg/L	351	58	931	57	1.02 (0.81-1.30)	0.68
> 5.56 mg/L	127	21	351	21	0.97 (0.73-1.29)	0.71

* The values listed across from the variable names represent the p-values for the entire model. The p-values listed across from each category within variables represent the p-values for that category.

Table 5. Distribution of variables between cases of congenital anomalies from the Annapolis Valley and controls from the Halifax Regional Municipality, as well as univariate associations between incidence of congenital anomalies and all variables for cases and controls (1988-2006).

	Cases from Annapolis Valley (n=606)		Controls from Halifax Regional Municipality (n=1301)		Cases from Annapolis Valley and Controls from Halifax Regional Municipality (n=1907)	
	n	%	n	%	Crude Odds Ratio (95% CI)	p-value
Sex						0.87
Female	268	48	636	49	1.0	
Male	285	52	665	51	1.02 (0.83-1.24)	0.87
Missing	53		0			
Season of conception						0.83
Winter	83	15	203	16	1.0	
Spring	139	25	308	24	1.10 (0.79-1.53)	0.50
Summer	162	29	370	28	1.07 (0.78-1.47)	0.72
Fall	169	31	420	32	0.99 (0.72-1.34)	0.52
Missing	53		0			
Year of Conception						0.93
1987-1991	83	15	204	16	1.0	
1992-1996	113	20	278	21	1.0 (0.71-1.40)	0.75
1997-2001	282	51	643	49	1.08 (0.81-1.44)	0.56
2002-2006	75	14	176	14	1.05 (0.72-1.52)	0.89
Missing	53		0			
Maternal age						0.57
< 20	50	9	99	8	1.21 (0.85-1.73)	0.38
20-34	435	79	1044	80	1.0	
≥ 35	68	12	158	12	1.03 (0.76-1.40)	**0.001**
Missing	53		0			
Parity						0.61
0	273	49	660	51	1.0	
1-2	252	46	588	45	1.04 (0.85-1.27)	0.55
3+	28	5	53	4	1.28 (0.79-2.06)	0.34
Missing	53		0			
Pre-pregnancy weight						0.67
< 50	44	9	90	7	1.19 (0.81-1.75)	0.37
50- 69	281	59	685	53	1.0	
≥ 70	155	32	375	29	1.01 (0.80-1.27)	0.56
Missing	126		151	12		
Smoker						**<0.0001**
No	358	59	923	71	1.0	
Yes	248	41	378	29	**1.69 (1.38-2.07)**	**<0.0001**
Diabetes						0.22
No	586	97	1273	98	1.0	
Gestational	15	2	24	2	1.36 (0.71-2.61)	0.68
Other Diabetes	5	1	4	0	2.71 (0.73-10.14)	0.22
Thyroid disease						0.94
No	604	100	1297	100	1.0	
Yes	2	0	40	0	1.08 (0.20-5.88)	0.94
Folate supplementation						**0.01**
No	111	58	275	48	1.0	
Yes	79	42	296	52	**0.66 (0.47-0.92)**	**0.01**
Missing	416		730			
Folate fortification						0.19
No	300	50	602	46	1.0	
Yes	306	50	699	54	0.88 (0.72-1.07)	0.19
Water source						
Surface	118	19	1301	100		
Ground	488	81	0	0		
Municipal water						
Yes	245	40	1301	100		
No	361	60	0	0		
Nitrate exposure level						
< 1 mg/L	127	21	1301	100		
1-5.56 mg/L	351	58	0	0		
> 5.56 mg/L	127	21	0	0		

* The values listed across from the variable names represent the p-values for the entire variable. The p-values listed across from each category within variables represent the p-values for that category. [a] Odds ratios could not be calculated using controls from HRM because there was no variability in the water quality and nitrate variables among controls from HRM.

Table 6. Distribution of risk factors, water quality variables and nitrate by maternal age group among cases of congenital anomalies (n=553) in Annapolis Valley (1988-2006). *

Variables	Maternal Age at Conception					
	< 20 (n=50)		20-34 (n=435)		≥ 35 (n=68)	
	n	%	n	%	n	%
Sex of fetus						
Female	20	44	218	50	30	44
Male	30	56	217	50	38	56
Season of conception						
Winter	6	12	69	16	8	12
Spring	14	28	106	24	19	28
Summer	14	28	130	30	18	26
Fall	16	32	130	30	23	34
Year of conception						
1987-1991	11	22	68	16	4	6
1992-1996	8	16	101	23	4	6
1997-2001	23	46	210	48	49	72
2002-2006	8	16	56	13	11	16
Parity						
0	**41**	**82**	**212**	**49**	**20**	**29**
1-2	**9**	**18**	**207**	**48**	**36**	**53**
3+	**0**	**0**	**16**	**4**	**12**	**18**
Smoker						
No	**15**	**30**	**295**	**68**	**48**	**71**
Yes	**35**	**70**	**140**	**32**	**20**	**29**
Diabetes						
None	49	98	418	96	66	97
Gestational	1	2	13	3	1	1
Other diabetes	0	0	4	1	1	1
Endocrine disorder						
No	50	100	433	100	68	100
Yes	0	0	2	0	0	0
Folic Acid Supplements						
No	13	87	79	54	19	63
Yes	2	13	66	46	11	37
Missing	35		290		38	
Pre-pregnancy weight (kg)						
< 50	11	24	22	6	11	19
50-70	31	67	223	59	27	47
> 70	4	9	131	35	20	34
Missing	6		59		10	
Folate fortification						
No	23	46	205	47	19	28
Yes	27	54	230	53	49	72
Water source						
Surface	16	32	82	19	8	12
Ground	34	68	353	81	60	88
Municipal water						
Yes	23	46	178	41	22	32
No	27	54	257	59	46	68
Nitrate exposure level						
< 1 mg/L	18	36	91	21	8	12
1-5.56 mg/L	22	44	257	59	44	65
> 5.56 mg/L	10	20	87	20	16	24

*Variables that are bolded are significantly different ($p < 0.05$) between groups.

Table 7. Multivariable associations between incidence of congenital anomalies and all variables for cases and controls from the Annapolis Valley (1987-2006). *

	Matching Variables (n=2188)	Matching and Demographic Variables (n=2188)	Matching, Demographic and Risk Variables (n=697)	Matching, Demographic, Risk, and Water Quality Variables (n=697)	Matching, Demographic, Risk, Water Quality, and Nitrate (n=697)
Matching Variables					
Sex of fetus					
Female	1.0	1.0	1.0	1.0	1.0
Male	0.99 (0.82-1.20)	0.99 (0.82-1.21)	1.21 (0.86-1.72)	1.22 (0.86-1.73)	1.21 (0.85-1.72)
Season of conception					
Winter	1.0	1.0	1.0	1.0	1.0
Spring	1.04 (0.76-1.43)	1.05 (0.76-1.44)	1.38 (0.74-2.56)	1.38 (0.74-2.57)	1.38 (0.74-2.58)
Summer	0.97 (0.72-1.34)	0.99 (0.72-1.34)	1.16 (0.63-2.13)	1.15 (0.62-2.12)	1.15 (0.62-2.13)
Fall	0.97 (0.72-1.32)	0.96 (0.72-1.27)	1.20 (0.65-2.23)	1.20 (0.65-2.23)	1.23 (0.66-2.29)
Year of conception					
1987-1991	1.0	1.0	--	--	--
1992-1996	0.98 (0.71-1.36)	0.97 (0.70-1.36)	1.0	1.0	1.0
1997-2001	1.00 (0.71-1.36)	0.96 (0.72-1.27)	1.13 (0.62-2.07)	1.13 (0.62-2.09)	1.18 (0.64-2.17)
2002-2006	1.05 (0.73-1.51)	1.02 (0.70-1.45)	0.95 (0.46-1.93)	0.95 (0.46-1.94)	1.00 (0.49-2.05)
Demographic Variables					
Maternal age					
< 20		1.01 (0.71-1.44)	0.78 (0.39-1.58)	0.78 (0.39-1.58)	0.78 (0.38-1.58)
20-34		1.0	1.0	1.0	1.0
≥ 35		**1.38 (1.01-1.88)**	1.28 (0.76-2.14)	1.27 (0.76-2.13)	1.26 (0.75-2.11)
Parity					
0		1.0	1.0	1.0	1.0
1-2		**0.73 (0.60-0.90)**	**0.66 (0.46-0.96)**	**0.66 (0.46-0.96)**	**0.68 (0.47-0.99)**
3+		0.68 (0.43-1.07)	0.67 (0.27-1.68)	0.68 (0.27-1.69)	0.67 (0.27-1.67)
Risk Factors					
Smoker			1.0	1.0	1.0
No			1.32 (0.88-2.00)	1.35 (0.89-2.04)	1.36 (0.89-2.05)
Yes					
Diabetes			1.0	1.0	1.0
No			1.31 (0.43-3.93)	1.30 (0.43-3.92)	1.25 (0.42-3.77)
Gestational			1.82 (0.29-	1.86 (0.30-	1.90 (0.30-
Other diabetes			11.47)	11.75)	11.96)
Thyroid disease					
No			1.0	1.0	1.0
Yes			--	--	--
Folic Acid Supplement					
No					
Yes			1.0	1.0	1.0
Pre-pregnancy weight			0.98 (0.68-1.42)	0.98 (0.68-1.42)	0.99 (0.68-1.43)
(Kg)					
< 50			1.08 (0.54–2.15)	1.08 (0.54–2.16)	1.06 (0.53–2.12)
50-69			1.0	1.0	1.0
≥ 70			0.94 (0.65-1.37)	0.95 (0.65-1.39)	0.94 (0.64-1.37)
Water Quality Variables					
Water source					
Surface				1.0	1.0
Ground				1.18 (0.66-2.11)	0.49 (0.13-1.77)
Municipal water					
Yes				1.0	1.0
No				0.88 (0.55-1.40)	0.87 (0.53-1.42)
Nitrate Exposure level					
< 1 mg/L					1.0
1-5.56 mg/L					2.70 (0.77-9.46)
> 5.56 mg/L					2.16 (0.58-8.12)
Log Likelihood P-Value	>0.99	0.26	0.74	0.82	0.71

* The first column lists all variables included in the study and all other columns display the results of a logistic regression model. Each model was constructed by adding one group of variables to the previous model to the left. The groups of variables included in each model are listed at the top of each column.

Table 8. Multivariable associations between incidence of congenital anomalies and all variables (except folic acid supplementation and pre-pregnancy weight) for cases and controls from the Annapolis Valley (1987-2006). *

	Matching Variables (n=2188)	Matching and Demographic Variables (n=2188)	Matching, Demographic, and Risk Variables (n=2188)	Matching, Demographic, Risk, and Water Quality Variables (n=2188)	Matching, Demographic, Risk, Water Quality and Nitrate Variables (n=2188)
Matching Variables					
Sex of fetus					
Female	1.0	1.0	1.0	1.0	1.0
Male	0.99 (0.82-1.20)	0.99 (0.82-1.21)	0.98 (0.81-1.20)	0.99 (0.81-1.20)	0.98 (0.81-1.20)
Season of conception					
Winter	1.0	1.0	1.0	1.0	1.0
Spring	1.04 (0.76-1.43)	1.05 (0.76-1.44)	1.05 (0.76-1.44)	1.05 (0.76-1.44)	1.05 (0.76-1.44)
Summer	0.97 (0.72-1.34)	0.99 (0.72-1.34)	1.00 (0.73-1.37)	1.00 (0.74-1.37)	1.00 (0.74-1.37)
Fall	0.97 (0.72-1.32)	0.96 (0.72-1.27)	0.97 (0.71-1.32)	0.97 (0.71-1.32)	0.98 (0.72-1.33)
Year of conception					
1987-1991	1.0	1.0	1.0	1.0	1.0
1992-1996	0.98 (0.71-1.36)	0.97 (0.70-1.36)	0.98 (0.71-1.37)	0.99 (0.71-1.38)	0.99 (0.71-1.38)
1997-2001	1.00 (0.71-1.36)	0.96 (0.72-1.27)	0.97 (0.73-1.29)	0.98 (0.74-1.31)	1.00 (0.75-1.34)
2002-2006	1.05 (0.73-1.51)	1.02 (0.70-1.45)	1.02 (0.71-1.48)	1.03 (0.72-1.49)	1.05 (0.72-1.52)
Demographic Variables					
Maternal age					
< 20		1.01 (0.71-1.44)	0.94 (0.66-1.35)	0.94 (0.66-1.35)	0.95 (0.66-1.36)
20-34		1.0	1.0	1.0	1.0
≥ 35		**1.38 (1.01-1.88)**	**1.40 (1.02-1.91)**	**1.39 (1.02-1.91)**	**1.40 (1.02-1.91)**
Parity					
0		1.0	1.0	1.0	1.0
1-2		**0.73 (0.60-0.90)**	**0.73 (0.59-0.89)**	**0.73 (0.59-0.89)**	**0.73 (0.60-0.90)**
3+		0.68 (0.43-1.07)	0.67 (0.43-1.06)	0.68 (0.43-1.07)	0.70 (0.43-1.06)
Risk Factors					
Smoker					
No			1.0	1.0	1.0
Yes			**1.28 (1.03-1.57)**	**1.28 (1.03-1.57)**	**1.27 (1.03-1.57)**
Diabetes					
No			1.0	1.0	1.0
Gestational			1.30 (0.70-2.41)	1.29 (0.70-2.40)	1.31 (0.71-2.44)
Other diabetes			0.97 (0.34-2.73)	0.97 (0.34-2.73)	0.96 (0.34-2.70)
Thyroid disease					
No			1.0	1.0	1.0
Yes			0.64 (0.14-3.00)	0.65 (0.14-3.05)	0.64 (0.14-3.01)
Water Quality Variables					
Water source					
Surface				1.0	1.0
Ground				0.85 (0.66-1.09)	0.70 (0.35 – 1.41)
Municipal water					
Yes				1.0	1.0
No				1.10 (0.81-1.51)	0.82 (0.63 – 1.07)
Nitrate Exposure level					
< 1 mg/L					1.0
1-5.56 mg/L					1.65 (0.83-3.27)
> 5.56 mg/L					1.66 (0.81-3.42)
Log Likelihood P-Value	>0.99	0.26	0.19	0.22	0.22
-2 Log Likelihood Ratio Test		**0.01**	0.20	0.45	0.32

* The first column lists all variables included in the study and all other columns display the results of a logistic regression model. Each model was constructed by adding one group of variables to the previous model to the left. The groups of variables included in each model are listed at the top of each column.

Table 9. Multivariable associations between incidence of congenital anomalies and all variables (except water quality and nitrate) for cases from the Annapolis Valley and controls from the Halifax Regional Municipality (1987-2006). * [a]

	Matching Variables (n=1854)	Matching and Demographic Variables (n=1854)	Matching, Demographic and Risk Variables (n=696)
Matching Variables			
Sex of fetus			
Female	1.0	1.0	1.0
Male	1.01 (0.83-1.24)	1.01 (0.83-1.24)	1.12 (0.79-1.60)
Season of conception			
Winter	1.0	1.0	1.0
Spring	1.10 (0.79-1.52)	1.10 (0.80-1.53)	1.35 (0.73-2.50)
Summer	1.06 (0.77-1.46)	1.07 (0.78-1.46)	1.20 (0.65-2.20)
Fall	0.98 (0.72-1.35)	0.99 (0.72-1.36)	1.35 (0.73-2.48)
Year of conception			
1987-1991	1.0	1.0	--
1992-1996	1.01 (0.72-1.42)	1.02 (0.72-1.42)	1.0
1997-2001	1.08 (0.81-1.44)	1.09 (0.81-1.46)	1.22 (0.66-2.28)
2002-2006	1.04 (0.72-1.51)	1.06 (0.72-1.53)	1.13 (0.55-2.35)
Demographic Variables			
Maternal age			
< 20		1.26 (0.87-1.82)	1.14 (0.54-2.40)
20-34		1.0	1.0
≥ 35		0.98 (0.72-1.35)	1.03 (0.62-1.72)
Parity			
0		1.0	1.0
1-2		1.08 (0.87-1.32)	0.91 (0.63-1.32)
3+		1.33 (0.81-2.18)	1.35 (0.50-3.69)
Risk Factors			
Smoker			
No			
Yes			1.0
Diabetes			1.24 (0.81-1.92)
No			
Gestational			1.0
Other diabetes			2.92 (0.84-10.13)
Thyroid disease			3.17 (0.42-24.01)
No			
Yes			1.0
Folic Acid Supplement			--
No			
Yes			1.0
Pre-pregnancy weight (Kg)			0.70 (0.48-1.02)
< 50			0.98 (0.48-1.98)
50-69			1.0
≥ 70			0.94 (0.64-1.37)
Log Likelihood P-Value	0.98	0.97	0.59

* The first column lists all variables included in the study and all other columns display the results of a logistic regression model. Each model was constructed by adding one group of variables to the previous model to the left. The groups of variables included in each model are listed at the top of each column.

[a] Odds ratios could not be calculated using controls from Halifax Regional Municipality because there was no variability in the water quality and nitrate variables among controls from HRM.

Table 10. Multivariable associations between incidence of congenital anomalies and all variables (except water quality, nitrate, folic acid supplementation and pre-pregnancy weight) for cases from the Annapolis Valley and controls from the Halifax Regional Municipality (1987-2006). * [a]

	Matching Variables (n=1854)	Matching and Demographic Variables (n=1854)	Matching, Demographic and Risk Variables (n=1854)
Matching Variables			
Sex of fetus			
Female	1.0	1.0	1.0
Male	1.01 (0.83-1.24)	1.01 (0.83-1.24)	1.01 (0.82-1.23)
Season of conception			
Winter	1.0	1.0	1.0
Spring	1.10 (0.79-1.52)	1.10 (0.80-1.53)	1.09 (0.78-1.51)
Summer	1.06 (0.77-1.46)	1.07 (0.78-1.46)	1.07 (0.78-1.47)
Fall	0.98 (0.72-1.35)	0.99 (0.72-1.36)	0.98 (0.72-1.34)
Year of conception			
1987-1991	1.0	1.0	1.0
1992-1996	1.01 (0.72-1.42)	1.02 (0.72-1.42)	1.04 (0.74-1.46)
1997-2001	1.08 (0.81-1.44)	1.09 (0.81-1.46)	1.11 (0.82-1.49)
2002-2006	1.04 (0.72-1.51)	1.06 (0.72-1.53)	1.08 (0.74-1.57)
Demographic Variables			
Maternal age			
< 20		1.26 (0.87-1.82)	1.14 (0.78-1.66)
20-34		1.0	1.0
≥ 35		0.98 (0.72-1.35)	1.00 (0.73-1.37)
Parity			
0		1.0	1.0
1-2		1.08 (0.87-1.32)	1.08 (0.87-1.33)
3+		1.33 (0.81-2.18)	1.28 (0.78-2.11)
Risk Factors			
Smoker			
No			1.0
Yes			**1.32 (1.06-1.65)**
Diabetes			
No			1.0
Gestational			1.52 (0.79-2.92)
Other diabetes			2.90 (0.77-10.95)
Thyroid disease			
No			1.0
Yes			1.14 (0.21-6.35)
Log Likelihood P-Value	0.98	0.97	0.54
-2 Log Likelihood Ratio Test		0.64	**0.04**

* The first column lists all variables included in the study and all other columns display the results of a logistic regression model. Each model was constructed by adding one group of variables to the previous model to the left. The groups of variables included in each model are listed at the top of each column.

[a] Odds ratios could not be calculated using controls from Halifax Regional Municipality because there was no variability in the water quality and nitrate variables among controls from Halifax Regional Municipality.

Table 11. Multivariable associations between incidence of congenital anomalies and all variables (except folic acid supplementation and pre-pregnancy weight), for all cases and controls from the Annapolis Valley, and for only cases and controls from the Annapolis Valley that have groundwater sources of drinking-water (1987-2006). [a]

	Cases and Controls from Annapolis Valley (n=2188)	Cases and Controls from Annapolis Valley Served by Groundwater (n=1778)
Matching Variables		
Sex of fetus		
Female	1.0	1.0
Male	0.98 (0.81-1.20)	1.07 (0.86-1.33)
Season of conception		
Winter	1.0	1.0
Spring	1.05 (0.76-1.44)	1.01 (0.70-1.45)
Summer	1.00 (0.74-1.37)	1.11 (0.78-1.57)
Fall	0.98 (0.72-1.33)	1.04 (0.74-1.48)
Year of conception		
1987-1991	1.0	1.0
1992-1996	0.99 (0.71-1.38)	1.02 (0.70-1.49)
1997-2001	1.00 (0.75-1.34)	1.12 (0.81-1.55)
2002-2006	1.05 (0.72-1.52)	0.97 (0.64-1.48)
Demographic Variables		
Maternal age		
< 20	0.95 (0.66-1.36)	0.86 (0.56-1.31)
20-34	1.0	1.0
≥ 35	**1.40 (1.02-1.91)**	**1.52 (1.08-2.13)**
Parity		
0	1.0	1.0
1-2	**0.73 (0.60-0.90)**	**0.77 (0.61-0.97)**
3+	0.70 (0.43-1.06)	**0.56 (0.35-0.99)**
Risk Factors		
Smoker		
No	1.0	1.0
Yes	**1.27 (1.03-1.57)**	**1.44 (1.14-1.82)**
Diabetes		
No	1.0	1.0
Gestational	1.31 (0.71-2.44)	1.10 (0.53-2.30)
Other diabetes	0.96 (0.34-2.70)	1.25 (0.37-4.27)
Thyroid disease		
No	1.0	1.0
Yes	0.64 (0.14-3.01)	0.80 (0.16-3.93)
Water Quality		
Municipal water		
Yes	1.0	1.0
No	0.82 (0.62-1.07)	0.81 (0.62-1.06)
Nitrate Exposure level		
< 1 mg/L	1.0	1.0
1-5.56 mg/L	1.65 (0.83-3.27)	1.72 (0.86-3.41)
> 5.56 mg/L	1.66 (0.81-3.42)	1.73 (0.74-3.57)
Log-likelihood p-value	0.22	0.07

[a] Models could not be created for participants served by surface water as all live in Kentville and therefore have the same nitrate exposure status.

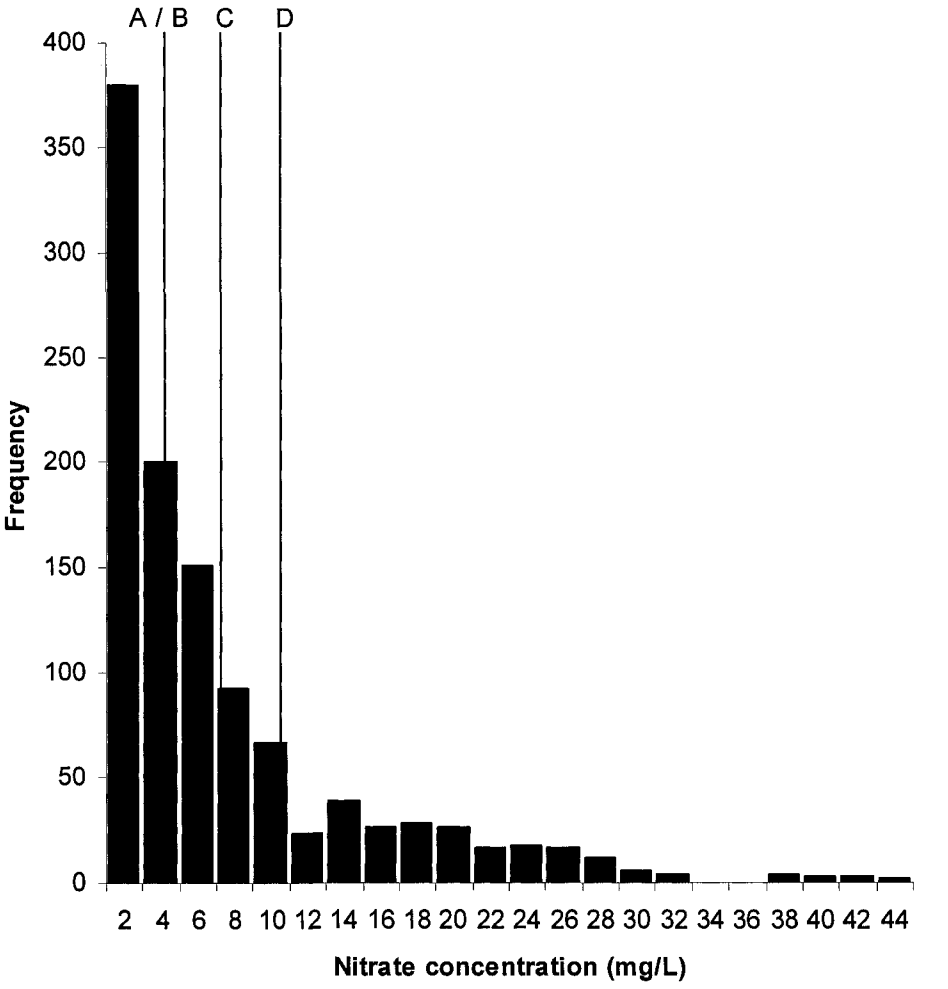
Table 12. Multivariable associations between incidence of congenital anomalies and all variables (except folic acid supplementation and pre-pregnancy weight), for all cases and controls from the Annapolis Valley, only cases and controls from the Annapolis Valley that live in municipal areas, and only cases and controls from the Annapolis Valley that live in rural areas.

	Cases and Controls from Annapolis Valley (n=2188)	Cases and Controls from Municipal Areas in Annapolis Valley (n=832)	Cases and Controls from Rural Areas in Annapolis Valley (n=1356)
Matching Variables			
Sex of fetus			
Female	1.0	1.0	1.0
Male	0.98 (0.81-1.20)	0.92 (0.67-1.26)	1.02 (0.79-1.31)
Season of conception			
Winter	1.0	1.0	1.0
Spring	1.05 (0.76-1.44)	1.15 (0.70-1.91)	0.96 (0.64-1.45)
Summer	1.00 (0.74-1.37)	1.16 (0.70-1.91)	0.91 (0.61-1.36)
Fall	0.98 (0.72-1.33)	1.11 (0.68-1.83)	0.90 (0.61-1.35)
Year of conception			
1987-1991	1.0	1.0	1.0
1992-1996	0.99 (0.71-1.38)	1.05 (0.64-1.73)	0.94 (0.60-1.47)
1997-2001	1.00 (0.75-1.34)	0.86 (0.55-1.34)	1.09 (0.74-1.60)
2002-2006	1.05 (0.72-1.52)	1.26 (0.71-2.22)	0.90 (0.55-1.48)
Demographic Variables			
Maternal age			
< 20	0.95 (0.66-1.36)	0.97 (0.57-1.67)	0.96 (0.59-1.56)
20-34	1.0	1.0	1.0
≥ 35	**1.40 (1.02-1.91)**	1.05 (0.61-1.80)	**1.65 (1.12-2.44)**
Parity			
0	1.0	1.0	1.0
1-2	**0.73 (0.60-0.90)**	**0.62 (0.45-0.87)**	0.81 (0.62-1.06)
3+	0.70 (0.43-1.06)	0.71 (0.34-1.51)	0.68 (0.38-1.22)
Risk Factors			
Smoker			
No	1.0	1.0	1.0
Yes	**1.27 (1.03-1.57)**	1.07 (0.76-1.50)	**1.40 (1.07-1.83)**
Diabetes			
No	1.0	1.0	1.0
Gestational	1.31 (0.71-2.44)	2.38 (1.01-5.62)	0.70 (0.14-3.26)
Other diabetes	0.96 (0.34-2.70)	1.33 (0.32-5.52)	0.68 (0.25-1.83)
Thyroid disease			
No	1.0	1.0	1.0
Yes	0.64 (0.14-3.01)	--	1.06 (0.21-5.40)
Water Quality			
Water source			
Surface	1.0	1.0	1.0
Ground	0.71 (0.35-1.43)	0.83 (0.38-1.83)	--
Nitrate Exposure level			
< 1 mg/L	1.0	1.0	1.0
1-5.56 mg/L	1.65 (0.83-3.27)	1.37 (0.62-3.03)	2.46 (0.55-10.91)
> 5.56 mg/L	1.66 (0.81-3.42)	--	2.44 (0.55-10.90)
Log-likelihood p-value	0.22	0.36	0.33

Table 13. Multivariable associations between incidence of congenital anomalies and all variables (except folic acid supplementation and pre-pregnancy weight), for cases and controls from the Annapolis Valley conceived prior to folate fortification (1987-1997), and for cases and controls from the Annapolis Valley conceived after folate fortification (1998-2006).

	Cases and Controls from Annapolis Valley Conceived from 1987-1997 (n=987)	Cases and Controls from Annapolis Valley Conceived from 1998-2006 (n=1201)
Matching Variables		
Sex of fetus		
Female	1.0	1.0
Male	1.0 (0.75-1.34)	0.96 (0.73-1.24)
Season of conception		
Winter	1.0	1.0
Spring	1.0 (0.61-1.63)	1.12 (0.73-1.71)
Summer	1.01 (0.62-1.63)	1.01 (0.67-1.53)
Fall	0.98 (0.62-1.55)	0.98 (0.64-1.50)
Year of conception		
1987-1991	1.0	--
1992-1996	0.96 (0.69-1.35)	--
1997-2001	0.94 (0.62-1.42)	1.0
2002-2006	--	1.02 (0.75-1.39)
Demographic Variables		
Maternal age		
< 20	0.85 (0.50-1.43)	1.01 (0.61-1.68)
20-34	1.0	1.0
≥ 35	1.35 (0.76-2.39)	**1.50 (1.03-2.19)**
Parity		
0	1.0	1.0
1-2	0.77 (0.56-1.05)	**0.68 (0.52-0.90)**
3+	0.75 (0.39-1.45)	0.59 (0.31-1.11)
Risk Factors		
Smoker		
No	1.0	1.0
Yes	1.01 (0.74-1.38)	**1.55 (1.16-2.06)**
Diabetes		
No	1.0	1.0
Gestational	1.63 (0.73-3.64)	2.27 (0.30-16.71)
Other diabetes	0.49 (0.06-4.15)	--
Thyroid disease		
No	1.0	1.0
Yes	1.55 (0.13-18.10)	0.36 (0.04-3.01)
Water Quality Variables		
Water source		
Surface	1.0	1.0
Ground	2.50 (0.62-10.08)	0.54 (0.23-1.29)
Municipal		
Yes	1.0	1.0
No	0.78 (0.53-1.14)	0.82 (0.56-1.20)
Nitrate Exposure level		
< 1 mg/L	1.0	1.0
1-5.56 mg/L	0.48 (0.10-1.60)	**2.44 (1.05-5.66)**
> 5.56 mg/L	0.47 (0.11-1.90)	2.25 (0.92-5.52)
Log-likelihood p-value	> 0.95	**0.04**

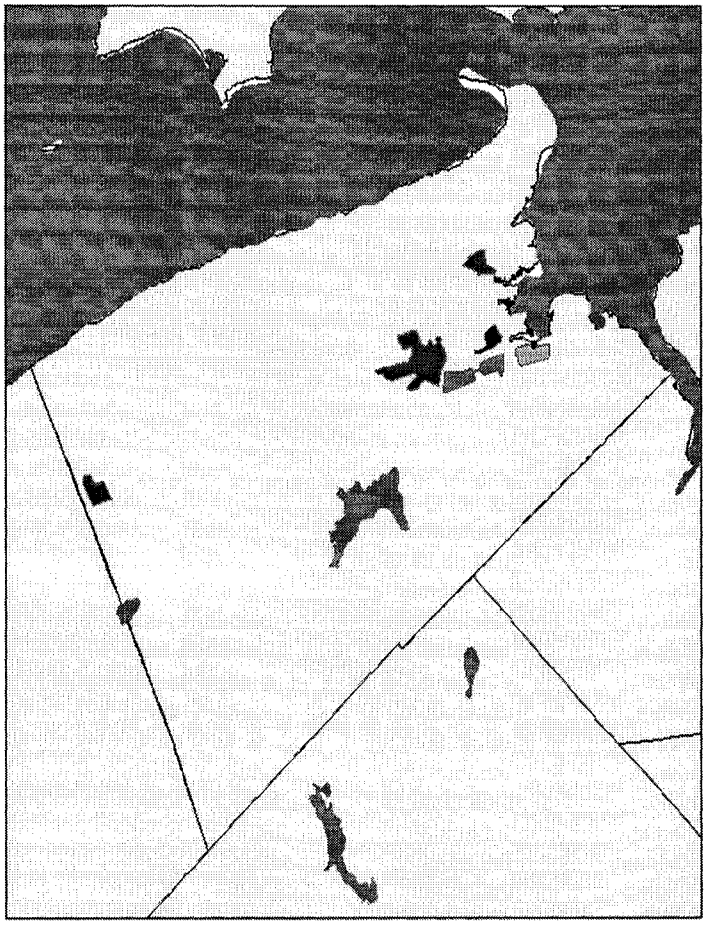
Figure 1. A histogram displaying nitrate concentrations (as nitrate-nitrogen) from all samples taken from rural wells in the Annapolis Valley study area from July 1999 to February 2000 (n=1113).



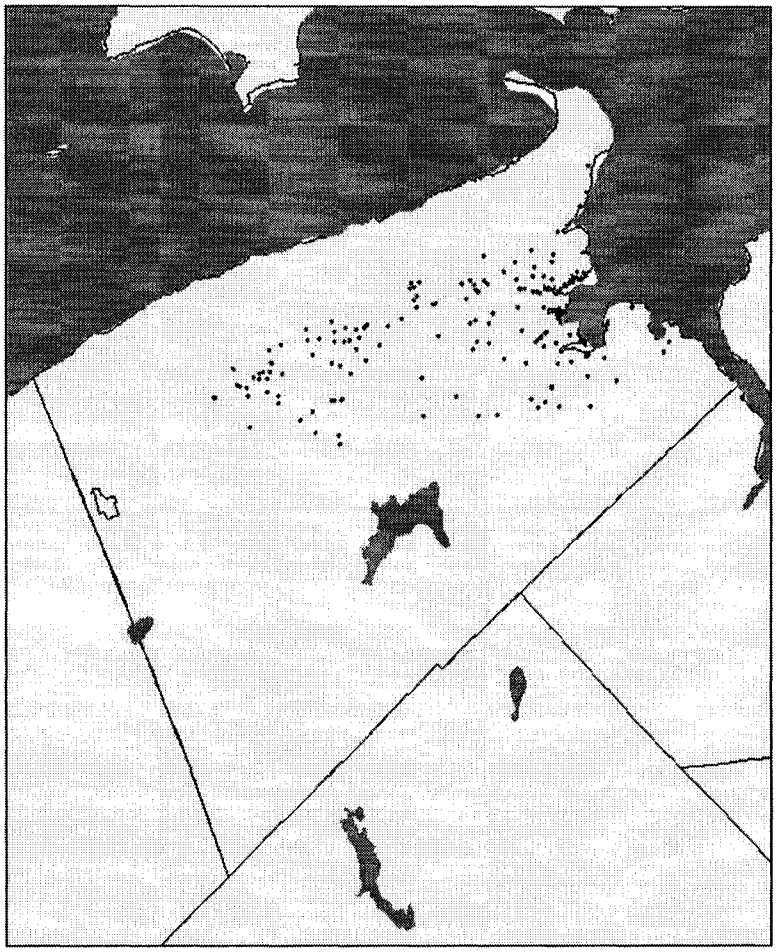
Legend:

A	Median nitrate concentration (3.79 mg/L)
B	Mean cube-root transformed nitrate concentration (3.72 mg/L)
C	Mean nitrate concentration (6.44 mg/L)
D	MAC (10 mg/L)

Map 1. The boundaries of the water distribution systems of municipalities with public water supplies within the Annapolis Valley study area.



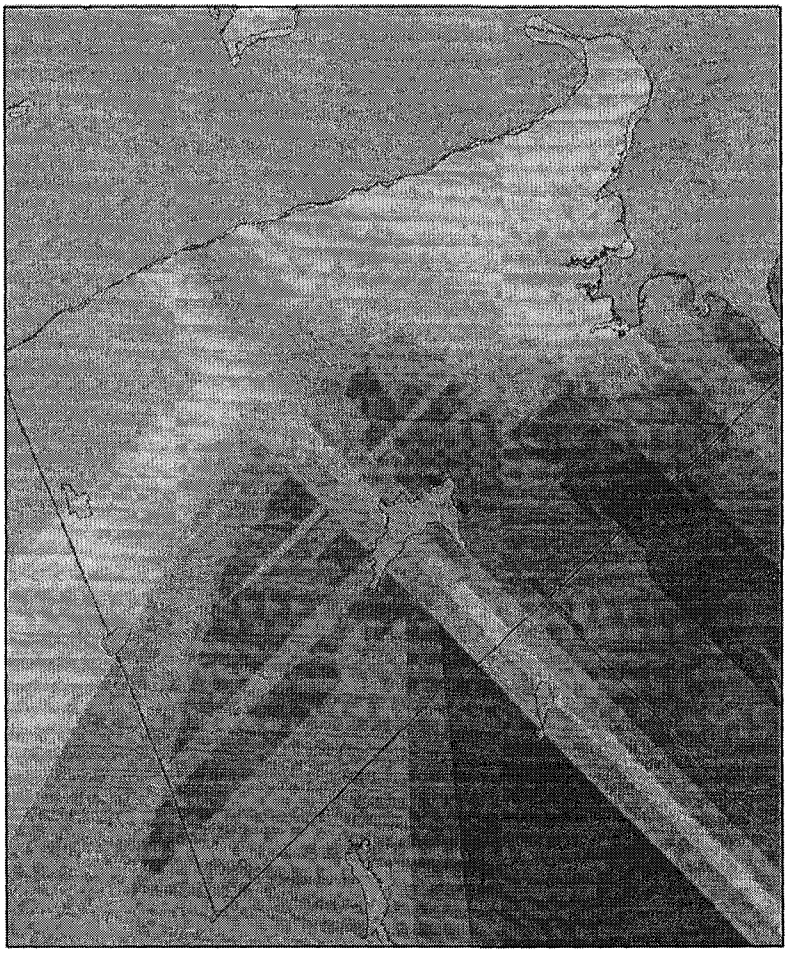
Map 2. The locations of 140 private wells in the Annapolis Valley study area between July 1999 and February 2000.



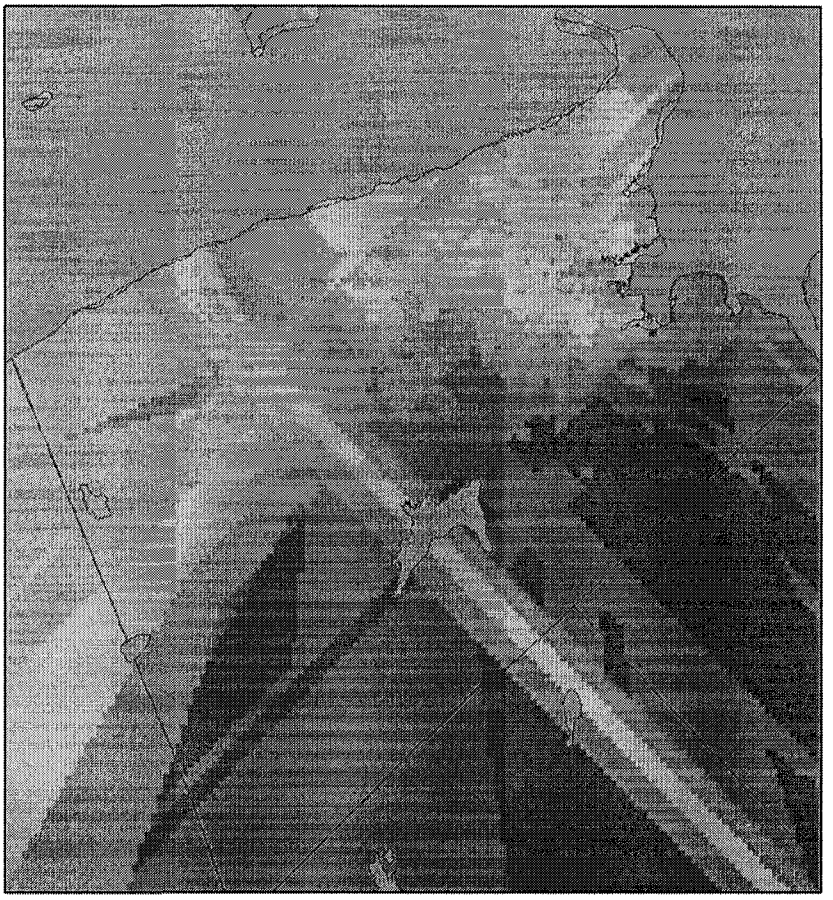
Legend

- Well

Map 3. Groundwater nitrate concentration estimates in the Annapolis Valley study area derived from an ordinary kriging interpolation of the nitrate concentrations measured from 140 private wells in the study area.



Map 4. Groundwater nitrate concentration estimates in the Annapolis Valley study area derived from an ordinary kriging interpolation of the nitrate concentrations measured from a randomly selected 90% of the 140 private wells in the study area.



Legend

Nitrate (mg/L)

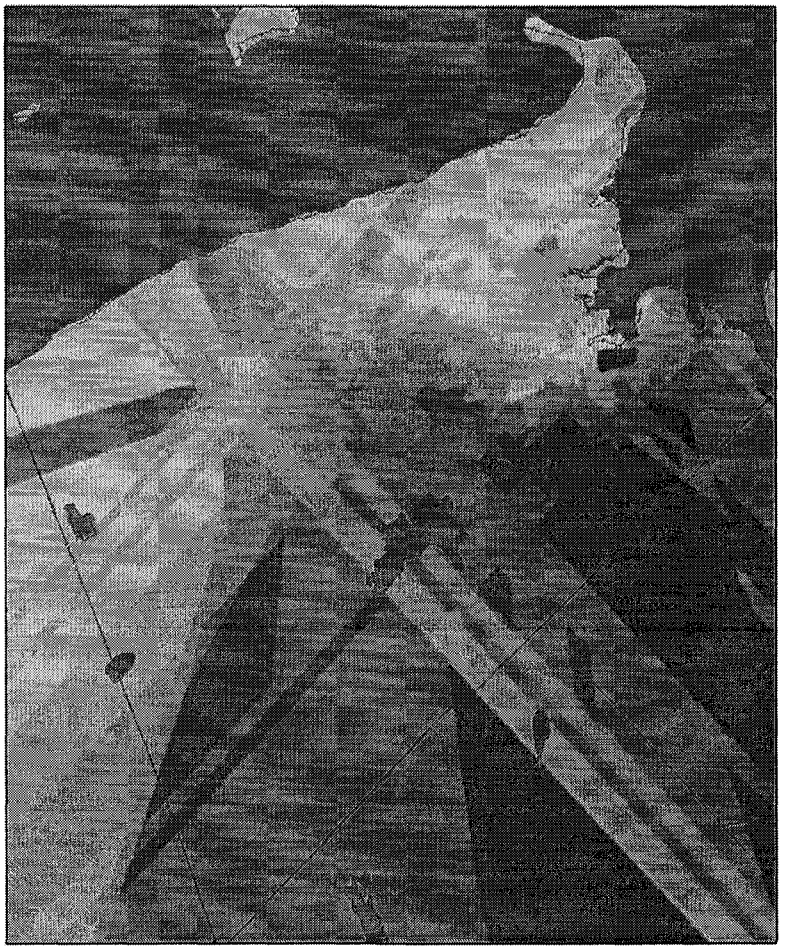
Prediction Map

[XYnitdb_training].[NITRATE]

Grid

0.00 - 1.00
1.00 - 2.00
2.00 - 3.00
3.00 - 4.00
4.00 - 5.00
5.00 - 5.56
5.56 - 6.00
6.00 - 7.00
7.00 - 8.00
8.00 - 9.00
9.00 - 10.00
10.00 - 13.00
13.00 - 16.00
16.00 - 19.00
19.00 - 22.00
22.00 - 25.00
25.00 - 30.00
30.00 - 35.00
35.00 - 40.81

Map 5. Drinking-water nitrate concentration estimates in the Annapolis Valley study area derived in rural areas from an ordinary kriging interpolation of the groundwater nitrate concentrations and derived in municipal areas from the median drinking-water nitrate concentrations of public water supplies.



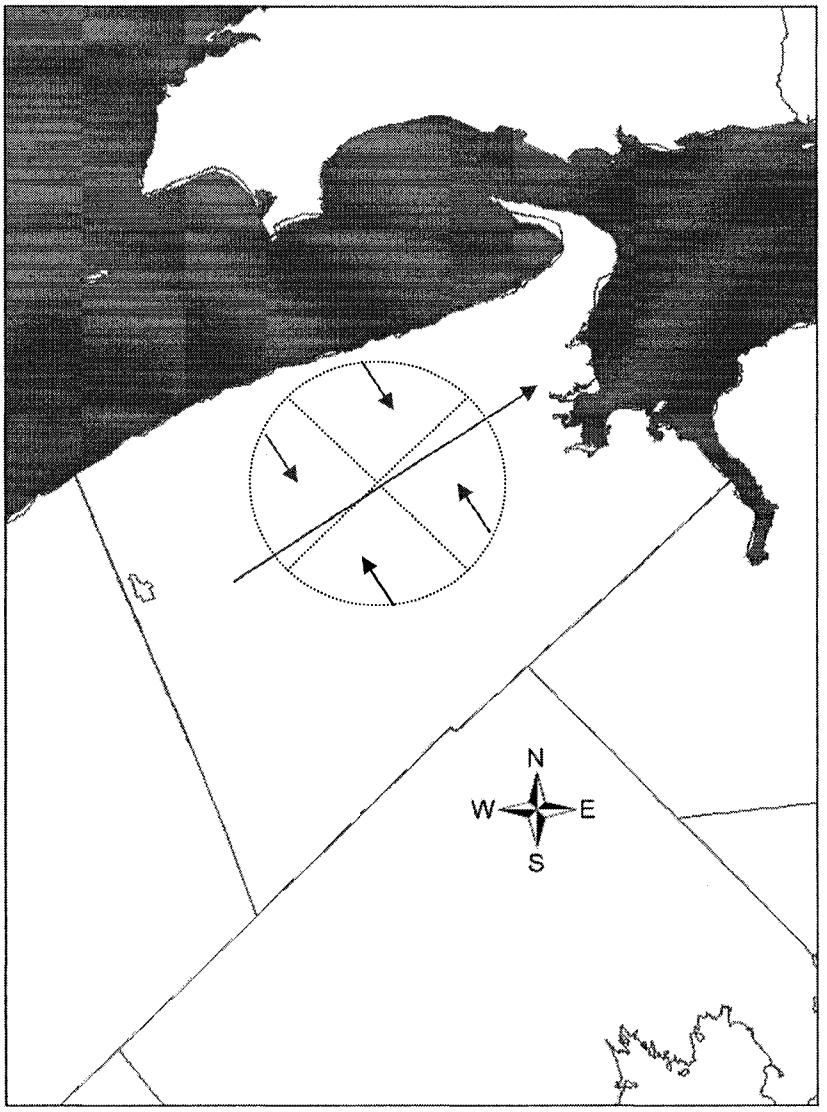
Legend

Nitrate (mg/L)

<VALUE>

0.38 - 1.00
1.01 - 2.00
2.01 - 3.00
3.01 - 4.00
4.01 - 5.00
5.01 - 5.56
5.57 - 6.00
6.01 - 7.00
7.01 - 8.00
8.01 - 9.00
9.01 - 10.00
10.01 - 13.00
13.01 - 16.00
16.01 - 19.00
19.01 - 22.00
22.01 - 25.00
25.01 - 30.00
30.01 - 35.00
35.01 - 40.00

Map 6. The direction of groundwater flow in the Annapolis Valley study area, and the shape of neighbourhood divided into quadrants from which neighbours were selected for the ordinary kriging interpolation.



APPENDIX 1

List of Major Anomalies by Body System as Defined by the Reproductive Care Program of Nova Scotia

Cardiovascular System:

- Absence pericardium/pericardial defect
- Acardia
- Aneurysm of vein of Galen
- Anomalous pulmonary venous return
- Aortic arch stenosis/ascending aorta stenosis
- Aortic valve stenosis
- Aortico-pulmonary window
- Arterio-venous mal of lung
- Asplenia
- Bicuspid aortic valve
- CHD, suspected
- CHD, type unknown
- CHD, unclassifiable
- Coarctation of the aorta
- Congenital cardiomyopathy
- Corrected left transposition
- Dextrocardia
- Double Outlet right ventricle
- Double aortic arch
- Double outlet left ventricle
- Dysplastic pulmonary valve
- Ebstein's malformation of tricuspid valve
- Endocardial cushion defect
- Endocardial fibroelastosis
- Hypoplastic left heart synd
- Insufficiency/cleft of mitral valve
- Interrupted aortic arch
- Intracardiac Mass
- Intrathoracic (Vascular) Ring
- Isolated ostium primum defect
- Isolated ostium secund defect
- Mitral atresia
- Mitral stenosis
- Patent ductus arteriosis
- Premature closure of foramen ovale
- Pseudotruncus
- Pulmonary artery atresia
- Pulmonary artery stenosis (pathologic)
- Pulmonary valve insufficiency

- Pulmonary valve stenos/atresia
- Pulmonary vein atresia
- Single atrium
- Single ventricle
- Tetralogy of Fallot
- Translocation great arteries/vessels
- Tricuspid atresia
- Tricuspid insufficiency
- Truncus arteriosus
- Ventricular septal defect

Central Nervous System:

- Agenesis of corpus callosum
- Anencephaly
- Arachnoid cyst
- Arhinencephaly
- Arthrogryposis/Contractures
- Brain Hypoplasia
- Cebocephaly
- Cerebellar hypoplasia
- Cerebro-retinal angiomatosis
- Cortical Dysplasia
- Cranium bifidum
- Cyclops
- Dandy-Walker Syndrome
- Dermal fistula
- Diastematomyelia
- Encephalocele
- Holoprosencephaly
- Hydranencephaly
- Hydrocephalus
- Lipomeningocele
- Lissencephaly
- Meningocele
- Meningomyelocele
- Moebius syndrome
- Neurofibromatosis
- Non-specific brain anomalies
- Pachygyria
- Polymicrogyria
- Rachischisis
- Schizencephaly
- Spina bifida
- Sturge-Webber
- Tuberous sclerosis

• Werdnig - Hoffmann Disease

Eye, Ear, Nose, Mouth, Throat:

• Aniridia
• Anophthalmia
• Branchial Cleft Anomaly
• Cataracts
• Central Blindness
• Choanal atresia
• Cleft Lip and/or Palate
• Corneal Opacities (congenital)
• Eyelid Fibrous Bands (Palpebral Fissure Band)
• Facial Cleft
• Glaucoma
• Hypoplastic Ears
• Laryngeal Atresia/Severe Congenital Laryngeal Stenosis
• Laryngeal Diverticulum
• Microphthalmia
• Microstomia
• Opacities Vitreous Humor/hyper Prim.vit
• Optic Atresia or Optic Nerve Hypoplasia
• Peter anomaly
• Radicular Cysts (Apex of Tooth
• Retinal Dysplasia
• Scleralization of cornea
• Stenosis/Atresia External Auditory Meatus/Canal
• Thyroglossal cyst

Gastrointestinal System:

• Alagilles' syndrome
• Annular pancreas
• Biliary Atresia
• Duplication of bowel
• Extrinsic Intestinal Obstruct
• Hepato-venous-occlusion disease liver
• Hirschsprung's disease
• Imperforate anus
• Intestinal Atresia
• Intestinal malrotation
• Intrinsic Intestinal Stenosis
• Meckel's Diverticulum
• Microcolon
• Microcolon-Megacystis-Hypoperistalsis Syndrome
• Paucity of intrahep bile duct

- Pyloric stenosis
- Tracheo-Esoph Fistula/Atresia
- Volvulus

Genitourinary System:

- Absent uterus/Fallopian tubes
- Agenesis of Bladder
- Agenesis/Hypoplasia/Atrophy Kidney
- Bicornuate uterus
- Bladder neck obstruction
- Cloacal exstrophy
- Congenital vaginal cyst
- Double Urinary System
- Double vagina
- Epispadias
- Exstrophy of Bladder
- Genital agenesis/hypoplasia
- Horseshoe kidney
- Hydronephrosis/Hydroureter/Renal Pelvis Distortion
- Hypoplasia of uterus
- Hypospadias Complex
- Imperforate hymen
- Large echodense kidneys,UNK
- Nephrotic syndrome
- Ovarian cyst
- Patent (persistent) urachus
- Pelvic Kidney
- Polycystic Kidney
- Posterior urethral valve
- Rectal-ano-urethral fistula
- Rectovaginal fistula
- Renal Dysplasia
- Torsion of ovary
- Torsion of testis
- Transposition of the scrotum
- Urachal cyst
- Ureteral atresia/stenosis
- Ureteral diverticulum
- Ureterocele
- Ureteropelvic junction obst
- Urethral obstruction
- Urogenital sinus

Inguinal Canal:

- Cryptorchidism
- Femoral Hernia
- Inguinal Hernia

Metabolic:

- Zellweger Syndrome

Multiple Anomalies due to Chromosomal Aberrations:

- 13Q- Syndrome
- 18 P- syndrome
- 18q- syndrome
- 2q+ syndrome
- 2q- syndrome
- 47Xy
- 4Q+ Syndrome
- 4q- syndrome
- 5 to 7 translocation
- 5q+ syndrome
- 6q+ syndrome
- 7 to 9 translocation
- 9q+ syndrome
- Chromosome 1p+
- Chromosome 9p+
- Chromosome Ring 13
- Chromosome Ring 14
- Chromosome Ring 15
- Cri-du-chat syndrome
- Deletion of part of # 14 chrom
- Down's Syndrome (trisomy 21)
- Extra material on P (# 15 chromos)
- Gonosomal intersex
- Klinefelters Syndome
- Marker chromosome (female)
- Marker chromosome (male)
- Mosaic 13 syndrome
- Mosaic Down's syndrome
- Mosaic Turner's syndrome
- Mosaic trisomy 12
- Prader-Willi Syndrome
- Ring 5
- Tetrasomy 12p
- Translocation 13
- Translocation 21
- Triploidy

- Trisomy 13
- Trisomy 14
- Trisomy 18
- Trisomy 19
- Trisomy 22
- Trisomy 7
- Trisomy 9
- Trisomy C group (incl tri 8)
- Turner's syndrome
- Unknown type
- Wolf syndrome
- X chromosome Q+
- XYY syndrome

Multiple Anomalies not due to Chromosomal Aberrations:

- Adams-Oliver syndrome
- Apert's syndrome
- Asplenia syndrome
- Beckwith's syndrome
- Body Stalk Anomaly
- Branchio-oto-renal syndrome
- Camptomelic syndrome
- Carpenter syndrome
- Charcot-Marie-Tooth Syndrome
- Charge association
- Cleido-cranial dysostosis
- Conradi's disease
- Cornelia De Lange syndrome
- DiGeorge syndrome
- Ectrodactyly-ectodermal dys
- Fetal alcohol syndrome
- Fetal hydantoin syndrome
- Fraser's syndrome
- Frontal-nasal dysplasia seq
- Goldenhar syndrome
- Holt Oram syndrome
- Hypomandibular faciocranial
- Klippel/Trenaunay/Weber syn
- Lowe's syndrome
- Marfan's syndrome
- Meckel-Gruber syndrome
- Multiple Pterygium syndrome
- Noonan syndrome
- Oromandibular limb hypogen syn
- Oto-facial-digital

- Otocephaly
- Pena Shokeir, type 1 phenotype
- Pena Shokeir, type 2 phenotype
- Pentalogy of Cantrell
- Phenocopy
- Pierre-Robin syndrome
- Poland syndrome
- Polysplenia syndrome
- Prune belly syndrome
- Rhizomelic dwarfism
- Roberts' syndrome
- Rubinstein-Taybi
- Russell-Silver syndrome
- Simpson-Golabi-Behemel synd
- Smith-Lemli-Opitz syndrome
- Stickler's syndrome
- Townes-Brock syndrome
- Treacher-Collins' syndrome
- Unclassifiable
- Vater association
- Walker-Warbury syndrome
- Williams' syndrome

Musculoskeletal System:

- Absence abdom wall
- Absence/Hypoplasia Pectoralis Major
- Absent ulna
- Achondroplasia
- Bifid thumb
- Camptodactyly
- Chondrodystrophy
- Claw hand,anoms hand/foot
- Club Foot
- Congenital Hip Dislocation
- Craniosynostosis/Cran'stenosis
- Crouzon's Disease
- Diastrophic dysplasia syndrome
- Dislocation of knee
- Dislocation of radial heads
- Epigastric hernia
- Fractures-cause unknown
- Gastroschisis
- Hemihypertrophy
- Hypoplastic calvaria
- Hypoplastic disease,small dig

- Iniencephalus
- Kleeblattschadel Syndrome
- Klippel-Feil syndrome
- Myasthenia gravis-newborn
- Myopathy
- Myotonic dystrophy
- Omphalocele
- Omphalomesenteric cyst
- Osteogenesis imperfecta
- Phocomelia/amelia/limb reduct
- Polydactyly
- Radial Aplasia/Hypoplasia
- Sacrococcygeal agenesis/bifid sacrum
- Short femur
- Sirenomelus
- Skull depression,unk etiology
- Sprengel's deform shoulder
- Syndactyly
- Thanatophoric dwarfism
- Torticollis
- Trigonocephaly
- Triphalangeal thumb
- Vertebral Anomalies

Oligohydramnios Syndrome

- Oligohydramnios, cause unk
- Potter's with oligohydramnios
- Potter's without oligohydram
- Urinary anomalies excluding renal agenesis

Respiratory System:

- Acinar dysplasia
- Bronchogenic cyst
- Diaphragmatic Hernia
- Hypoplasia of Diaphragm
- Pulmonary Hypoplasia/Agenesis
- Pulmonary Sequestria
- Pulmonary hyperplasia
- Tracheal agenesis
- Tracheal atresia

Skin:

- Absent Breasts
- Amniotic Bands Deformity/Syndrome
- Anhidrotic ectodermal dysplasia

- Bullous, type Unknown
- Cutis Hyperelastica
- Cutis Laxa
- Cutis aplasia
- Cutis marmorata congenital
- Epidermolysis bullosa
- Goltz syndrome
- Ichthyosis
- Incontinentia pigmenti
- Non-bullous Dermatosis, type unknown
- Urticaria pigmentosa